

A two part study to investigate the morbidity and mortality of long-term tamoxifen therapy in women with breast cancer and the effects of exposure to exogenous oestrogens in women with benign and malignant breast disease.

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DECLARATION

I hereby declare that this thesis was written entirely by myself and that all the work reported herein was performed by myself, except where the contribution of colleagues is acknowledged.

Signed.

CAROLYN McDONALD

Date..... 08 | 12 | 99 **.....**

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THE UNIVERSITY OF EDINBURGH

ABSTRACT OF THESIS

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Title of Thesis... A two part study to investigate the morbidity and mortality of long-term tamoxifen therapy
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malignant breast disease.....

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The study uses data linkage methodology to investigate the effects of exogenous exposure to endocrine agents in women with malignant and benign breast disease. The study is in two parts:

Part A: Tamoxifen is widely used as first-line endocrine therapy in the treatment of breast cancer and women may be exposed to many years of treatment. The aim of this part of the study was to add to the body of evidence available on the risk to benefit profile of long-term tamoxifen use. The method was linkage of the Scottish adjuvant tamoxifen trial database with Scottish hospital inpatient statistics (SMR1), cancer registration (SMR6) and death records from the General Registry Office (GRO). The Scottish adjuvant tamoxifen trial recruited 1323 patients between 1978 and 1984 and includes women who have received up to 18 years of tamoxifen therapy. The data linkage enabled ascertainment of causes of admission to hospital, registration of new primary cancers and causes of death in the trial population to the end of March 1996. Analysis by Cox Proportional Hazards method considered tamoxifen status as 'never use', 'ever use' or 'current use'. The results confirm a reduced incidence of ischaemic heart disease and increased thromboembolism. An increased risk of cataract in tamoxifen users was also detected. The effect of long-term tamoxifen therapy on the incidence of new primary malignancies and fractures is also discussed.

Part B: The Edinburgh Breast Unit database has a record for every woman presenting to the department. The details recorded include previous exposure to exogenous oestrogens in the form of hormone replacement therapy (HRT) and oral contraception (OC). Many prospective epidemiological studies have looked at the effects of long-term exposure to exogenous oestrogens, mostly using annual or biennial questionnaires to determine events, with the inevitable problems of incomplete ascertainment. Data linkage in Scotland provides an alternative means of ascertaining events and offers an opportunity to study the effects of oestrogen exposure on a range of pre-defined endpoints. The aim of this part of the study was to determine the risk of death from cardiovascular disease and incidence of new primary cancers by exposure status to exogenous oestrogen, using case-control studies nested within the population presenting to the EBU between 1989 and 1994. Analysis was by conditional logistic regression. The problem of missing data reduced the power of the study. This is discussed and suggestions for further study are proposed.

CONTENTS

Title Page

Declaration

Acknowledgements

Abstract

Contents

Glossary

List of Tables

List of Figures

CHAPTER 1

Rationale and Objectives - Part A

1.1 Tamoxifen morbidity and non breast cancer mortality.....	1
1.2 The Scottish Adjuvant Tamoxifen Trial	3
1.3 Rationale	4
1.4 Objectives	6

Rationale and Objectives - Part B

1.5 Evidence of other health effects of oestrogen exposure...	8
1.6 Edinburgh Breast Unit database	8
1.7 Rationale	9
1.8 Objectives	9

PART A

Morbidity of long-term tamoxifen therapy within the Scottish adjuvant tamoxifen trial

CHAPTER 2

Tamoxifen: clinical development and mode of action

2.1 Background	11
2.2 Metabolism	13
2.3 Clinical studies in breast cancer.....	15
2.4 Duration of treatment	16
2.5 Tamoxifen in combination with other therapies.....	18
2.6 Primary systemic therapy	20
2.7 Breast cancer treatment in elderly patients	21
2.8 Chemoprevention	22
2.9 Mechanisms of tamoxifen action	24
2.10 Tamoxifen resistance	29

CHAPTER 3	Other Health Effects of Tamoxifen	
3.1	Cardiac effects.....	31
3.2	Thromboembolic effects	36
3.3	Carcinogenic effects	39
3.4	Other Effects on the Endometrium	48
3.5	Skeletal Effects	49
3.6	Ocular Effects.....	58
 CHAPTER 4	 Record Linkage and Data Acquisition	
4.1	Record Linkage System	61
4.2	Confidentiality and Data Protection	64
4.3	Linkage with the Scottish tamoxifen trial data	64
4.4	Linkage Validation	72
4.5	Linkage updates	78
4.6	Ascertainment of 'Events'	79
4.7	Analysis Database	82
4.8	Trial Follow-up	84
4.9	Glossary	88
 CHAPTER 5	 Statistical Methods – Part A	
5.1	Overview of statistical methods	89
5.2	Statistical methods in the current study.....	103
5.3	Preparation of data for analysis.....	108
5.4	Implementation of the Cox model.....	109
5.5	Statistical analyses	111
5.6	Expression of results	112
5.7	Supplementary analyses	112
 CHAPTER 6	 Results – Part A	
6.1	Validation of matched records in linked dataset	114
6.2	Tamoxifen trial follow-up	116
6.3	Ascertainment of 'Events'.....	116
6.4	Statistical Analyses	124
6.5	Other failure events.....	137
6.6	Supplementary analyses.....	138
 CHAPTER 7	 Discussion – Part A	
7.1	Statistical Aspects	141
7.2	Risk/Benefit assessment of tamoxifen	151
7.3	Myocardial infarction and ischaemic heart disease	152
7.4	Thromboembolic disease	154
7.5	Cerebrovascular disease.....	155
7.6	New primary malignancies	156
7.7	Effects on the skeleton	160
7.8	Ocular effects.....	162
7.9	Benign endometrial disease.....	165
7.10	Dementias.....	166
7.11	The tamoxifen life-cycle.....	167

PART B

Effect of exposure to exogenous oestrogens on mortality and incidence of new primary cancers

CHAPTER 8	8.1 Introduction.....	170
	8.2 Epidemiological studies of exogenous oestrogen use.....	171
	8.3 Hormone Replacement Therapy (HRT).....	172
	8.4 Oral Contraception (OC).....	178
CHAPTER 9	Case-control Study: Methods and Analysis	
	9.1 Types of epidemiological studies.....	183
	9.2 Methodology of case-control studies	187
	9.3 Analysis of observational studies	193
	9.4 Design of current study.....	197
	9.5 Data linkage.....	204
	9.6 Statistical methods.....	211
	9.7 Changes to the study design	214
CHAPTER 10	Results – Part B	
	10.1 Results of linkage of EBU data with SMR6 and GRO records.....	215
	10.2 Number of events.....	215
	10.3 Scope of the current study.....	217
	10.4 Data validation.....	218
	10.5 Overview of results of data collection.....	219
	10.6 Power of the study	222
	10.7 Results for ischaemic heart disease.....	224
	10.8 Results for thromboembolism	225
	10.9 Results for endometrial cancer.....	226
CHAPTER 11	Discussion – Part B	
	11.1 Study power: comparison with published studies.....	228
	11.2 Recent published data of oestrogen exposure and cardiovascular disease.....	230
	11.3 Suggestions for future work.....	231
CHAPTER 12	Final Discussion	
	12.1 Study Conclusions.....	234
	12.2 New agents for hormone replacement therapy.....	235
	12.3 Selective Estrogen Receptor Modulators (SERMs).....	236
	12.4 SERMs as oestrogen replacement therapies.....	236
	12.5 Triphenylethylene derivatives.....	240
	12.6 Benzothiophene derivatives.....	241
	12.7 Conclusions.....	243

APPENDICES

- Appendix 1** McDonald CC et al. (1991) *BMJ* **303**;435-437
- Appendix 2** McDonald CC et al. (1995) *BMJ* **311**;977-980
- Appendix 3** Microsoft Access SQL queries
- Appendix 4** Structure of Tambase database
- Appendix 5** Microsoft Access update queries
- Appendix 6** Sample of case-control data for ischaemic heart disease prepared for conditional logistic regression analysis
- Appendix 7** Sample of case-control data for thromboembolism prepared for conditional logistic regression analysis

BIBLIOGRAPHY

GLOSSARY

4-OHT	4 hydroxytamoxifen
ASCII	American Standard Code for Information Interchange
ATAC	'Adjuvant Tamoxifen Alone or in Combination'
ATTom	'Adjuvant Tamoxifen Treatment-Offer More?'
BCPT	US Breast Cancer Prevention Trial
BMD	Bone mineral density
cAMP	Cyclic adenosine monophosphate
CHD	coronary heart disease
CI	confidence interval
CMF	cyclophosphamide, methotrexate, 5-fluorouracil
CVA	cerebrovascular accident
CRC	Cancer Research Campaign
DMBA	dimethylbenzanthracene
df	degrees of freedom
DNA	deoxyribose nucleic acid
DVT	deep vein thrombosis
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EBU	Edinburgh Breast Unit
EORTC	European Organisation for the Research and Treatment of Cancer
ER	Oestrogen Receptor
ERE	Oestrogen Response Element
FDA	Food and Drug Administration
fmol/mg	fentimoles per milligram
GRO	General Registry Office
HAF	hepatocellular altered foci
HDL	high density lipoprotein
HPV	human papilloma virus
HR	hazard ratio

Glossary

HRT	hormone replacement therapy
hx	hysterectomy
IBIS	International Breast Cancer Intervention Study
IGF	Insulin-like growth factor
ICRF	Imperial Cancer Research Fund
IHD	Ischaemic heart disease
ISD	Information and Statistics Division
ITT	Intent to treat
LDL	low density lipoprotein
LH-RH	luteinizing hormone-releasing hormone
LMP	last menstrual period
LRS	Likelihood Ratio Statistic
M	molar
mdr	multidrug resistance
mg	milligram
MI	myocardial infarction
MORE	Multiple Outcomes of Raloxifene
MS	Microsoft
NATO	Nolvadex Adjuvant Trial Organisation
N-dMT	N-desmethyltamoxifen
NSABP	National Adjuvant Surgical Breast & Bowel Project
NYSIIS	New York State Intelligence Information System
OC	oral contraception
OR	odds ratio
ox	oophorectomy
p	probability
PAC	Privacy Advisory Committee
PE	pulmonary embolism
PKC	protein kinase C
PTH	parathyroid hormone

Glossary

PI	Pulsatility Index
RCT	Randomised controlled trial
RR	relative risk
RUTH	Raloxifene Use for the Heart
SCTO	Scottish Cancer Trials Office
SCTN	Scottish Cancer Therapy Network
SD	Standard deviation
SEER	Surveillance, Epidemiology and End Results
SMR	Scottish Morbidity Record
SMR1	Scottish hospital discharge records
SMR6	Scottish cancer registration records
SQL	Structured Query Language
STAR	Study of Tamoxifen and Raloxifene
TGF	Transforming growth factor
TOES	Tamoxifen Evaluation Study
UICC	International Union against Cancer
UKCCCR	United Kingdom Co-ordinating Committee for Cancer Research
μM	micromolar

LIST OF TABLES

Table 3.1	Relative risk of endometrial cancer in some large adjuvant tamoxifen trials	44
Table 4.1	Database field names and description for importing the linked dataset	67
Table 4.2	Endpoints and their ICD9 classification, for the study of morbidity within the Scottish adjuvant tamoxifen trial.....	80
Table 4.3	Key variables on analysis database (Tambase) imported from original trial database.....	83
Table 5.1	Survival times (months) for women in breast cancer study.....	91
Table 5.2	Calculation of Kaplan-Meier estimate of survivor function for two groups of sample data in <i>Table 5.1</i>	93
Table 5.3	Calculation of log rank statistic for data in <i>Table 5.1</i>	97
Table 5.4	Additional variables created in the analysis database.....	107
Table 5.5	Description of tamoxifen status and illustration of switching between time dependent variables t0 and t1.....	109
Table 6.1	Number and percentage of manually validated records with matching scores of 17-23 inclusive	115
Table 6.2	Total number of events identified from the validated, linked dataset, by ICD9 classification and randomised option	118
Table 6.3	Fractures identified in the validated, linked dataset, by ICD9 classification of site and randomised option.....	120
Table 6.4	New primary malignancies identified in the validated, linked dataset, by ICD9 classification of site and randomised option	121
Table 6.5	Ocular events identified in the validated, linked dataset, By ICD9 classification and randomised option	123
Table 6.6	Log-rank analysis comparing the survival experience of the tamoxifen group with the observation group, as randomised.....	125

Table 6.7	Hazard Ratios and 95% Confidence Intervals for specified outcomes by randomised option, ever/never, and current/not current use of tamoxifen, using the proportional hazards method...135
Table 6.8	Hazard Ratios and 95% Confidence Intervals for specified outcomes by adding randomised option, ever/never and current/not current use of tamoxifen to a model containing age as a continuous variable, using the proportional hazards method137
Table 6.9	Tamoxifen status, duration of exposure and time since stopped in 11 cases of endometrial cancer.....138
Table 6.10	Results of comparison of incidence of ocular endpoints, with no censoring at metastatic relapse, between the randomised groups, ever/never and current/not current use of tamoxifen.....139
Table 6.11	Results of comparison of incidence of fracture between the randomised groups, ever/never and current/not current use of tamoxifen, adjusted for the presence or absence of metastatic disease.....140
Table 7.1	Summary of risk to benefit profile of tamoxifen in healthy women and those with breast cancer.....168
Table 9.1	Causes of death endpoints for study.....198
Table 9.2	Sites of new primary malignancy as endpoints.....199
Table 9.3	Variables extracted from EBU database for linking with SMR6 and FRO records205
Table 9.4	Structure of linked dataset showing records from 3 different sources – EBU, SMR6, GRO207
Table 9.5	Structure of database for entering exposure data obtained From EBU database.....209
Table 9.6	Description of calculated fields for the case-control analyses.....212
Table 10.1	Number of events identified from linkage of EBU data with GRO records216
Table 10.2	Number of new primary malignancies at all sites (excluding breast) identified from linkage of EBU data with SMR6 records....216
Table 10.3	Number of new primary malignancies at sites of interest for the current study.....217
Table 10.4	Data inconsistencies identified in EBU database.....218

Table 10.5	Number of cases available for analysis on the EBU database.....	220
Table 10.6	Overview of data collected from EBU database.....	221
Table 10.7	Minimum sample size required in terms of detectable odds ratio for exposed compared with non exposed individuals for different correlation coefficients of exposure between cases and controls.....	223
Table 10.8	Numbers of exposed, non exposed and exposure unknown cases and controls for the endpoint of ischaemic heart disease.....	224
Table 10.9	Numbers of exposed, non exposed and exposure unknown cases and controls for the endpoint of thromboembolism.....	226

LIST OF FIGURES

Figure 2.1	Chemical structure of <i>cis</i> and <i>trans</i> forms of tamoxifen	12
Figure 2.2	Potential ligands produced by tamoxifen metabolism	14
Figure 4.1	Sample output from linked data imported into MS Access database.....	68
Figure 4.2	Sample output from linked dataset after structural modification of the MS Access database.....	73
Figure 4.3	Flow diagram for validation of linked records in tamoxifen trial and combined databases	76
Figure 5.1	Schematic representation of censoring	90
Figure 5.2	Survival curve for data in <i>Table 5.1</i>	95
Figure 6.1	Survival curves showing rate of MI in the two randomised groups	126
Figure 6.2	Survival curves showing rate of IHD (other than MI) in the two randomised groups	127
Figure 6.3	Survival curves showing rate of PE/DVT in the two randomised groups.....	128
Figure 6.4	Survival curves showing rate of CVA in the two randomised groups.....	129
Figure 6.5	Survival curves showing rate of new primary cancers in the two randomised groups.....	130
Figure 6.6	Survival curves showing rate of fracture in the two randomised groups.....	131
Figure 6.7	Survival curves showing rate of ocular outcomes In the two randomised groups	132
Figure 7.1	Cumulative log hazard for randomised groups with respect to MI.....	143
Figure 7.2	Cumulative log hazard for randomised groups with respect to other IHD (other than MI).....	144

Figure 7.3	Cumulative log hazard for randomised groups with respect to PE/DVT.....	145
Figure 7.4	Cumulative log hazard for randomised groups with respect to CVA	146
Figure 7.5	Cumulative log hazard for randomised groups with respect to new primary malignancies.....	147
Figure 7.6	Cumulative log hazard for randomised groups with respect to fractures.....	148
Figure 7.7	Cumulative log hazard for randomised groups with respect to ocular toxicity.....	149
Figure 7.8	Structure of toremifene.....	159
Figure 7.9	Structure of ICI 182780.....	159
Figure 9.1	Design of a cohort study	183
Figure 9.2	Design of a case-control study.....	184
Figure 9.3	Design of a nested case-control study.....	186
Figure 9.4	Schematic representation of confounding.....	190
Figure 9.5	Calculation of the Relative Risk and Odds Ratio	194
Figure 9.6	Comparison of OR and RR in a cohort study where disease is frequent.....	195
Figure 9.7	Comparison of OR and RR in a cohort study where disease is rare.....	196
Figure 9.8	Schematic representation of populations from which Cases and controls were selected.....	202
Figure 12.1	The structure of some triphenylethylene derivatives.....	238
Figure 12.2	The structure of a benzothiophene derivative.....	242

Part A**Morbidity of long-term tamoxifen therapy within the Scottish adjuvant tamoxifen trial****1.1 Tamoxifen morbidity and non breast cancer mortality**

Tamoxifen is now included in the treatment of choice for the majority of women diagnosed with breast cancer and is widely prescribed as adjuvant therapy after local treatment (surgery, radiotherapy). Only those women whose primary tumours exhibit no, or very low levels of, oestrogen receptor protein appear to gain little benefit from treatment. A number of trials have been initiated to determine whether tamoxifen has a role to play in the chemoprevention of breast cancer in women at high risk of the disease and these are described in *Chapter 2, Section 2.8*. The US Breast Cancer Prevention Trial demonstrated a 49% reduction in risk of invasive breast cancer in the tamoxifen group compared with the placebo group (Fisher, 1998) but this result has not so far been confirmed in other studies which are still ongoing

The acute side effects of tamoxifen treatment are well known. They are generally mild and include flushing and weight gain. Less is known about the late morbidity associated with long-term exposure to tamoxifen. A study of hospital admissions in patients randomised within the Stockholm trial of adjuvant tamoxifen found little difference in intercurrent mortality between tamoxifen and control patients. The total number of hospital admissions was similar for both groups, with significantly fewer admissions for immunologic diseases, such as thyroid disease, in the tamoxifen group (RR = 0.4, 95% CI: 0.2-0.9). There were non-significant increases in thromboembolic events and benign gynaecological disease in the tamoxifen group. However, the median follow-up in the study was only 4.5 years with a maximum exposure to tamoxifen (40 mg daily) of two years (Fornander et al., 1991). A later analysis, with a median follow-up of 6 years, showed a significantly reduced incidence of hospital admission due to cardiac disease (RR= 0.68, 95% CI: 0.48 –

0.97). The results favoured a longer duration of tamoxifen treatment (5 years as opposed to 2 years) (Rutqvist & Mattson, 1993).

A study of 153 breast cancer patients who had participated in two Cancer Research Campaign trials of adjuvant tamoxifen were recruited into a study of the long-term effects of tamoxifen exposure. Cholesterol levels were significantly lower, some hormone levels significantly higher and bone density was non significantly higher in current users. However, results in ex-users suggested that these effects are reversible on the cessation of treatment (Cuzick, 1993).

Published data on non breast cancer causes of death in women receiving tamoxifen therapy are scarce. The 1995 overview of randomised trials of tamoxifen therapy in early breast cancer, carried out on behalf of the Early Breast Cancer Trialists' Collaborative Group, included an analysis of non breast cancer mortality (Early Breast Cancer Trialists' Collaborative Group, 1998). The underlying causes of death that were not due to breast cancer were subdivided into 10 categories; endometrial cancer, other neoplastic, cardiac, cerebrovascular, pulmonary embolus, other vascular, respiratory, infective, other medical and non-medical causes. A significant difference in mortality between tamoxifen and control groups was evident only for endometrial cancer.

Two published studies, in which this author was closely involved, have investigated non breast cancer morbidity and mortality within the Scottish adjuvant tamoxifen trial. The first showed a reduction in fatal myocardial infarction in women randomised to receive adjuvant tamoxifen compared with the control group (McDonald & Stewart, 1991) (*Appendix 1*). The second study involved linkage of trial data with Scottish inpatient hospital statistics and provided evidence of a significantly decreased risk of hospital admission due to myocardial infarction and a significantly increased risk of admission due to thromboembolism associated with tamoxifen use (McDonald et al., 1995) (*Appendix 2*). The Scottish adjuvant tamoxifen trial is described in detail in *Section 1.2*

Although the available evidence indicates that tamoxifen has a beneficial oestrogenic effect on ischaemic heart disease and the skeletal system, data continue to point to the carcinogenic potential of tamoxifen. As the issue of optimal duration of treatment has not yet been settled, the clinical decision to prescribe tamoxifen over a prolonged period continues to be based on an analysis of risk versus benefit. Furthermore, tamoxifen was licensed in the US in November 1998 for reducing the incidence of breast cancer in healthy women and is now being prescribed to women who are considered at high risk of developing the disease.

It is therefore important to be able to define the risks and benefits of tamoxifen therapy as precisely as possible and the objective of this study is to make a contribution to the available body of evidence and help to clarify the risk-benefit profile of tamoxifen therapy.

1.2 The Scottish Adjuvant Tamoxifen Trial

Between 1978 and 1984, the Scottish adjuvant tamoxifen trial recruited 1323 patients who were undergoing mastectomy for operable breast cancer. Randomisation was for adjuvant tamoxifen, 20 mg daily for at least 5 years, or no adjuvant systemic therapy but tamoxifen to be given for at least six weeks on local or systemic relapse of disease. A key feature of the trial was the secondary randomisation, whereby those patients who were alive, free from disease and still receiving tamoxifen therapy 5 years from randomisation, were re-randomised at this time to stop treatment or to continue indefinitely. Consequently, to the end of March 1996, there are patients within the study who have had continuous exposure to tamoxifen of up to eighteen years. Prolonged durations of tamoxifen exposure are also seen in long-surviving recurrent patients in the observation arm, who started treatment at the time of relapse.

Patients eligible for inclusion in the study were those under 80 years of age with early breast cancer (T1-3, N0-1, M0 by the UICC TNM Classification of Malignant Tumours, Third Edition, 1978) suitable for mastectomy. Premenopausal women with involved axillary nodes were excluded and were, instead, considered for entry into

another Scottish study comparing chemotherapy and oophorectomy (Scottish Cancer Trials Breast Group and ICRF Breast Unit, 1993). Patients were stratified at the time of randomisation by node status (involved, not involved, unknown), menstrual status (premenopausal, postmenopausal), geographical source (Aberdeen, Dundee, Edinburgh, Glasgow) and initial local therapy. Local therapy was either mastectomy with axillary node clearance and no postoperative radiotherapy or mastectomy with axillary node sample. Patients having a node sample received postoperative radiotherapy if there was histological involvement of the axillary nodes and patients in whom there was no histological assessment of the nodes were randomised to receive, or not receive, postoperative radiotherapy.

Eleven patients who were recognised, within one month of entry, to be major violators of the eligibility criteria, were excluded from the analyses. The first analysis of trial data involving 1312 evaluable patients was published in 1987 and showed a highly significant delay in first relapse and in overall survival in the adjuvant arm of the trial (Breast Cancer Trials Committee, 1987).

1.3 Rationale

There are a number of unique factors, related to the Scottish adjuvant tamoxifen trial and to the hospital record system in Scotland, that combine to make the current study feasible and offer a valuable opportunity to add significantly to the available data on long-term tamoxifen morbidity.

Firstly, there are a number of key features in the design of the Scottish adjuvant tamoxifen trial:

- i) This was the first randomised tamoxifen study in which patients were randomised to receive 5 years of adjuvant therapy and those in the control arm received delayed tamoxifen on disease recurrence. Other studies were looking at one or two years of treatment.

- ii) The secondary randomisation procedure, after 5 years of treatment, to stop tamoxifen or continue indefinitely, has resulted in patients having exposures to tamoxifen of up to eighteen years. An analysis in 1998 showed that 354 women had received adjuvant tamoxifen for 5 years or more and 171 of these had received treatment for 10 years or more (Stewart, 1998 Personal Communication).
- iii) Patients were carefully followed-up annually, at the anniversary of their mastectomy, and strenuous efforts were made by the staff of the Scottish Cancer Trials Office (SCTO), under the direction of Dr Helen Stewart, to obtain follow-up information on all patients with the result that very few individuals have been lost to follow-up. Seven patients were lost before routine annual follow-up stopped in 1993 and a further sixteen have their last follow-up information prior to 1996.
- iv) The design of the study is such that patients' tamoxifen status can change during the course of the study for a number of different reasons including starting tamoxifen on disease recurrence. Data collection for the trial included obtaining details of tamoxifen starting and stopping dates. It is therefore possible to classify individuals according to their tamoxifen status – never received therapy, received therapy and stopped, currently receiving therapy - throughout the entire follow-up period.

The second unique facility which provides the basis for the current study is the availability in Scotland of a well-developed record linkage system, whereby a permanently linked data set of Scottish health related data – containing all hospital discharge data, cancer registrations and General Registry Office death records from 1981 to 1995 – can be linked with each other and with external data sets of various types. This procedure is carried out at the Information and Statistics Division of the

Scottish Health Service in Edinburgh (ISD) and provides a very powerful tool for studying morbidity and mortality.

1.4 Objectives

Two studies related to the incidence of cardiovascular mortality and morbidity in the Scottish adjuvant trial have already been published (*Appendices 1 and 2*). The second of these studies utilised the linkage of trial data with hospital discharge data to the end of 1992. The objectives of the current study were to:

- i) Develop robust methods of handling the large quantity of electronic data received from ISD
- ii) Update the analysis of cardiovascular morbidity, using trial follow-up and hospital discharge data to the end of March 1996
- iii) Include information from cancer registration (SMR6) and General Registry Office (GRO) death records in the linkage with trial data. The previous study had linked only with hospital inpatient statistics (SMR1).
- iv) Extend the scope of the earlier studies to look at a range of other relevant, pre-defined morbidities within the trial population. Outcomes of interest for the current study included the incidence of hospital admissions for fractures, benign endometrial lesions, ocular problems and the incidence of new primary malignancies.

- v) Analyse the data to obtain point estimates of the relative risk of the specified endpoints, associated with tamoxifen use, together with 95% confidence intervals. An important feature of the analyses is the inclusion of tamoxifen status as a time-varying covariate, to reflect the changes occurring in a patient's tamoxifen status, between 'ever', 'never' and 'current' use, throughout the follow-up period.

Part B**Effect of exposure to exogenous oestrogens on mortality and incidence of new primary cancers****1.5 Evidence of other health effects of oestrogen exposure**

Women are widely exposed to exogenous oestrogens, in the form of oral contraception for premenopausal women and hormone replacement therapy for postmenopausal women. For many years, investigators have been looking at the evidence pertaining to the risks and benefits of such treatments. Studies investigating these issues in the past have generally been large, prospective epidemiological studies involving thousands of women being followed-up for many years. Ascertainment of outcomes is usually by questionnaire and the problem of obtaining complete follow-up information increase as the study duration increases. Flagging patients is an alternative means of determining cause of death and cancer incidence.

Current evidence suggests that postmenopausal hormone therapy reduces the risk of osteoporosis and cardiovascular disease and premenopausal hormone use increases the risk of thromboembolic disease. Reports on the effect of oestrogen use on the development of malignant tumours are inconsistent, with a reduction in the risk colorectal cancer, a reduction in ovarian cancer in premenopausal women and an increase in breast cancer incidence in postmenopausal women having been described. The literature describing the effects of exogenous oestrogen exposure is reviewed in *Chapter 8*.

1.6 Edinburgh Breast Unit database

A clinical database for the collection of details of patient demography, clinical history, diagnosis, treatment and follow-up has been in existence at the Edinburgh Breast Unit for 10 years. All women presenting to the Unit since 1989 have their details entered onto this database, which therefore contains records pertaining to women with breast cancer and benign breast disease.

Information relating to previous and current oestrogen use, as oral contraception or hormone replacement therapy, are recorded on the database at the time of the patient's presentation to the Unit and these details are also available in the hospital case-notes. Patients' notes are held within the Unit for malignant cases or within the main hospital medical records section for discharged patients with a diagnosis of benign disease.

1.7 Rationale

The facility available in Scotland to link an external database to inpatient hospital records, cancer registration and death records has already been mentioned in *Section 1.3*. This, combined with the extensive database of all patients presenting to EBU, offers a unique opportunity to investigate the effect of exposure to exogenous oestrogens on mortality and cancer incidence within a large population of pre and postmenopausal women. The advantage of data linkage methodology over conventional methods of questionnaire and interview for such a study is the ability to get complete ascertainment of the outcomes being studied. The current study has been designed as a nested case-control study within the historical cohort of EBU patients.

1.8 Objectives

The objectives of this observational study were to determine whether previous and current exposure to exogenous oestrogens, as oral contraception or hormone replacement therapy, is associated with:

- i) An altered death rate from specific causes, other than breast cancer. The causes of death to be studied were those for which published data were available and were defined as all ischaemic heart disease, including myocardial infarction, cerebrovascular disease and thromboembolic disease as determined by pulmonary embolism and deep vein thrombosis.

- ii) An altered incidence of second primary cancers. In the first instance, all second primary cancers, other than breast cancer, were to be ascertained. Breast cancer was excluded because of the problems associated with differentiating new primary cancers from previously diagnosed breast cancer in this population and because of the inherent bias in ascertaining breast cancer incidence in a population of women presenting to a breast disease clinic. The sites of new primary malignancy selected for further analysis would depend on the number of cases available and whether a possible association of incidence with oestrogen exposure had been hypothesised in the literature.

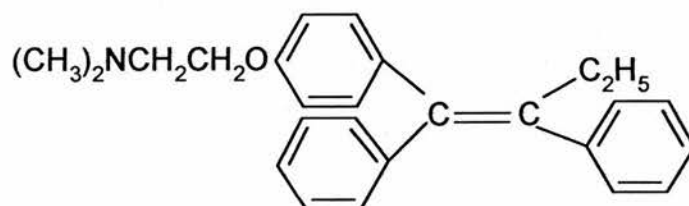
For both of these objectives, data linkage between the EBU database and cancer registration records (SMR6) and General Registry Office (GRO) death records would be used to identify cases with the outcomes of interest. Controls, who had not experienced these outcomes would be selected from the EBU database. Oestrogen exposure of both cases and controls, together with other relevant baseline information, would then be obtained by examination of available hospital data sources.

2.1 Background

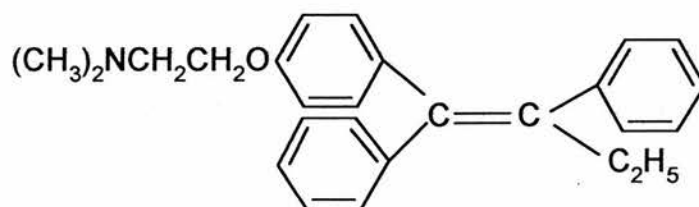
Tamoxifen, originally referred to as ICI 46474, is the *trans* isomer of 1(p-beta-dimethylamino ethoxy-phenyl)-1,2-diphenylbut-1-ene, a derivative of triphenylethylene (*Figure 2.1*). It was first synthesised in 1963 and found to be a potent antioestrogen in several, but not all, mammalian species. The alkylaminoethane side chain is essential for its antioestrogenic activity. Harper and Walpole were the first to compare the properties of the *trans* isomer with those of the oestrogenic *cis* isomer (Jordan et al., 1977). Tamoxifen was initially developed as a possible anti-fertility agent in the 1960's but was found, instead, to promote fertility and it is in the field of hormone-dependent cancer that this class of compounds has developed a key role.

Preclinical studies in rats demonstrated that the principal mode of action as an anti-oestrogen was specific antagonism of the effect of oestrogen at the level of the steroid receptors within the target tissue, by competition for the same receptor sites (Murphy et al., 1991). However, it became apparent that tamoxifen exhibited a species-specific pharmacology showing only agonist activity in the mouse, partial agonist/antagonist activity in the rat and antagonist activity only in the chick oviduct (Jordan et al., 1980). This selective stimulatory/inhibitory action was also shown in human tissue. Transplantation of human endometrial and breast cancer cells on either side of an athymic mouse and administration of tamoxifen, resulted in a stimulation of the growth of the endometrial cancer while the growth of the breast cancer was inhibited (Gottardis et al., 1988). In this model, tamoxifen was acting as an oestrogen to stimulate growth of the endometrial cancer and an antioestrogen to inhibit growth of the breast cancer. The agonist effects of tamoxifen may, in part, be explained by a significant increase in circulating oestrogen levels seen clinically in women with breast cancer after long-term administration of tamoxifen. This may also represent a mechanism for the development of drug resistance (Lum et al., 1997).

Figure 2.1



ICI 47699 cis isomer



ICI 46474 trans isomer

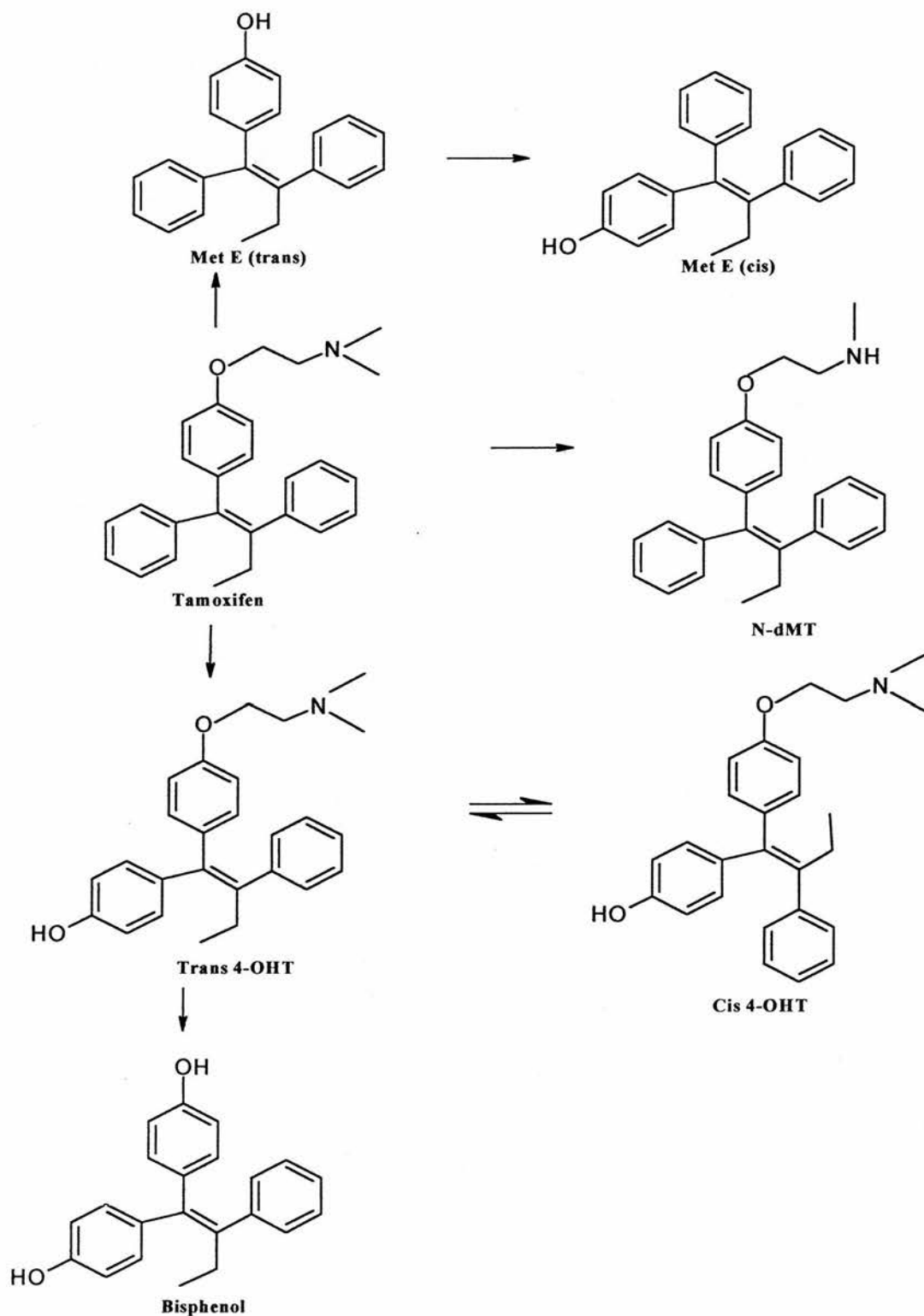
Chemical structure of *cis* and *trans* forms of 1(p-beta-dimethylamino ethoxy-phenyl)-1,2-diphenylbut-1-ene

Further work by Jordan on the pharmacology of tamoxifen in laboratory rats, demonstrated that treatment resulted in a dose-related decrease in the appearance and numbers of rat mammary tumours, 30 days after induction with dimethylbenzanthracene (DMBA). Oestrogen and progesterone receptor rich tumours responded more favourably to treatment but only continuous therapy maintained animals in a tumour free state, indicating that the action of tamoxifen is cytostatic rather than cytotoxic (Jordan et al., 1980).

2.2 Metabolism

The major metabolite of tamoxifen is N-desmethyltamoxifen (N-dMT), which is a weak antioestrogen. Other metabolic pathways produce metabolite E and bisphenol, which are oestrogenic (*Figure 2.2*). Para hydroxylation of the phenyl ring on carbon 1 of but-1-ene is a minor metabolic pathway, but the product, 4-hydroxytamoxifen (4-OHT), has a very high binding affinity for the oestrogen receptor and is intrinsically 100 times more active as an oestrogen receptor antagonist than is the parent drug (Jordan et al., 1977). This is due to the location of the hydroxyl group in an equivalent position to the 3-phenolic hydroxyl of 17 beta-oestradiol. A subsequent extensive study of structure-activity relationship has shown that this structure is optimal for the production of a potent antioestrogen (Murphy et al., 1991). 4-OHT metabolite has a less potent antitumour action in rats than the parent compound and this is probably due to its rapid clearance in this species (Jordan et al., 1980). The hydroxylation reaction is catalysed by human cytochrome P450 in liver microsomes and work is ongoing trying to identify the specific isoforms responsible for the catalytic action (Crewe et al., 1997). It has been demonstrated that 4-hydroxytamoxifen forms free radicals more rapidly than tamoxifen and causes much greater DNA damage. These radical mechanisms may play a role in the carcinogenic effects of tamoxifen on the endometrium and other target organs (Davies et al., 1997).

Figure 2.2



Potential ligands produced by tamoxifen metabolism (Tonetti & Jordan, 1995)

2.3 Clinical studies in breast cancer

Trials of tamoxifen in the palliation of advanced breast cancer started in 1969 and the first clinical paper showing evidence of its effectiveness was published in 1971 (Cole et al., 1971). In this study, 10/46 patients (22%) showed regression of their tumours. A review of subsequent studies in advanced disease during the 1970's and 1980's, involving 5,353 patients in 86 major clinical studies, demonstrated a 34% response rate with mean or median durations of response varying between 2 months and more than 24 months. Tamoxifen dose was between 20 mg and 40 mg daily (Litherland & Jackson, 1988). This overview, deriving data from 33 publications, noted the high tolerability of tamoxifen. The side effects of treatment appeared to be unrelated to dosage and resulted in discontinuation of therapy in less than 3% of patients. Longer duration of therapy did not result in increased adverse event reporting. The most frequent toxicity was gastrointestinal disturbance, including nausea and vomiting (10%) and hot flushes (10%-20%). More recently, data accrued from 200 subjects participating in the pilot feasibility study of tamoxifen for the prevention of breast cancer, coordinated by the Royal Marsden Hospital in London, showed that compliance to medication was high and the same for both tamoxifen and placebo groups. There was relatively low acute toxicity in both groups, with only hot flushes occurring in significantly more women receiving tamoxifen (27%) than in the placebo group (11%) (Powles et al., 1989). However, a prospective study of 161 patients with advanced breast cancer carried out at the Edinburgh Breast Unit found significant differences in hot flushes, weight gain and vaginal discharge in patients compared with controls. These symptoms were frequently described by the patients as 'worrying', and serve to emphasise that any side effects of treatment should be of concern (Ray & Leonard, 1996).

In 1973, tamoxifen was licensed in the UK as Novaldex® and, in 1977, clinical trials of adjuvant tamoxifen therapy in non-metastatic, operable breast cancer started. Many such studies, using a variety of tamoxifen regimens were initiated in Europe and the first overview analysis by the Early Breast Cancer Trialists' Collaborative Group was

reported in 1988 (Early Breast Cancer Trialists' Collaborative Group, 1988). A total of 16,500 women, in 28 randomised, controlled trials of adjuvant tamoxifen with up to 5 years of follow-up, were included and a reduction in the annual odds of death, in women over 50 years, of 20% was seen. A further analysis was carried out in 1990, by which time data were available on 30,000 women entered into clinical trials of tamoxifen. This analysis confirmed highly significant reductions in the annual rates of recurrence (25% SD 2) and death (17% SD 2) with the protective effect extending out to 10 years in spite of treatment being of much shorter duration (most commonly 2 years) (Early Breast Cancer Trialists' Collaborative Group, 1992).

Although the first overview analysis demonstrated a significant benefit in survival only in those women aged at least 50 years, the later evidence suggests that both pre and postmenopausal women benefit from treatment, with the 1990 data showing that tamoxifen produces a highly significant delay in recurrence in women under 50 ($p < 0.001$). These data counter early expectations that tamoxifen treatment may stimulate breast tumour growth in premenopausal women by inhibiting feedback control mechanisms resulting in high levels of circulating oestrogen. The proportional risk reductions are similar for both node positive and node negative disease with an absolute risk reduction greater in the node positive group. The most recent overview data (Early Breast Cancer Trialists' Collaborative Group, 1998) suggest that for women with ER negative tumours, the use of adjuvant tamoxifen requires further research, but for 30,000 women with ER positive or ER untested tumours, adjuvant tamoxifen treatment substantially improves the 10-year survival. Proportional reductions in breast cancer recurrence and in mortality appeared to be largely unaffected by other patient characteristics or treatments.

2.4 Duration of treatment

The optimal duration of treatment is still an unresolved question. Results, published in 1987, from the NSABP B-09 trial in which women received tamoxifen, in addition to

a chemotherapy regimen of melphalan plus fluorouracil, suggested that the addition of a third year of tamoxifen was beneficial (Fisher et al., 1987). Few data were available in the 1990 overview on tamoxifen durations exceeding two years, but indirect comparisons, of one year or less compared with two or more years, suggest that the longer durations of treatment are more effective in delaying death, with an indication, in a very small number of patients, that 5 years of treatment may be better still (Early Breast Cancer Trialists' Collaborative Group, 1992). Preliminary results from the Cancer Research Campaign trial, evaluating tamoxifen in women aged 50 years and over, suggest that 5 years of treatment may be better than two, with a significant delay in the time to relapse for patients receiving treatment for the longer time (Current Trials Working Party of the CRC Breast Cancer Trials Group, 1996). The Swedish Breast Cancer Cooperative Group also published results in 1996 demonstrating a significant advantage of five years of treatment compared with two, in postmenopausal women with oestrogen-positive tumours (defined in this study as greater than or equal to 0.1fmol/ μ g DNA) (Swedish Breast Cancer Co-operative Group, 1996). In the overview data published in 1998, there was considerably more evidence available from trials of 5 years or more of tamoxifen and a highly significant trend towards greater effect with longer treatment was seen ($2p < 0.00001$) (Early Breast Cancer Trialists' Collaborative Group, 1998). The proportional reductions in recurrence in 30,000 women during 10 years of follow-up were 21% (SD 3), 29% (SD 2) and 47% (SD 3) respectively for trials of 1 year, 2 years and 5 years of adjuvant tamoxifen respectively.

Although these results all concur with the view that longer is better, studies investigating more than 5 years of tamoxifen treatment do not demonstrate such definitive results. Data published from the NSABP B-14 trial for women with primary operable breast cancer, no axillary involvement and tumours with an oestrogen receptor content of 10 or more fmol/mg protein, showed significant benefit in disease-free and overall survival in women randomised to receive tamoxifen. This benefit

persisted through 10 years of follow-up but no additional benefit was seen in continuing therapy for more than 5 years (96% survival for those who discontinued tamoxifen after 5 years and 94% for those who continued active treatment) (Fisher et al., 1996). The report suggests that more than 5 years' adjuvant treatment is not warranted in routine clinical practice. The results of the comparison of 5 years adjuvant tamoxifen treatment with continuous therapy within the Scottish adjuvant tamoxifen trial suggest no additional benefit in extending therapy beyond 5 years and show a trend towards an increased incidence of endometrial carcinoma in those receiving prolonged treatment (Stewart et al., 1996).

With no clear direct evidence of a benefit of long term therapy, and the possibility of an increase in morbidity resulting from large cumulative doses of tamoxifen, a number of clinical trials are currently underway to directly compare the effect of different durations of tamoxifen therapy. The aTTom, trial, organised by the UKCCCR and coordinated at the CRC Trials Unit in Birmingham, randomises patients who have completed at least 2 years adjuvant tamoxifen therapy and who are disease-free, to continue treatment for at least a further 3 years or to stop therapy (Rea et al., 1998). The trial started in 1991 and aims to recruit 8,000 women to determine whether there is an overall survival benefit from extending the duration of therapy with adjuvant tamoxifen. The principal criterion for entry into the study is that the treating clinician is unsure whether or not to continue tamoxifen treatment. As of September 1998 just over 2,900 women had been randomised (UKCCR Register of Cancer Trials, www.ctu.mrc.ac.uk/ukcccr).

2.5 Tamoxifen in combination with other therapies

The issue of whether additional benefit can be derived by the addition of tamoxifen treatment to polychemotherapy was first addressed by the 1990 EBCTCG overview (Early Breast Cancer Trialists' Collaborative Group, 1992). The assessment of this combined modality treatment is complex, but the data suggested that tamoxifen and

chemotherapy act independently and that their effects are approximately additive. Further data which were accrued in the 1995 EBCTCG overview, show that chemotherapy plus about 5 years of tamoxifen was substantially better than the same chemotherapy alone, with mortality reductions of 39% (SD 22) and 49% (SD 10) for age less than 50 and more than 50 respectively (Early Breast Cancer Trialists' Collaborative Group, 1998). However, the report emphasises that this is not statistically reliable evidence, even with such a large dataset and that, as yet, there are no results available from large, directly randomised comparisons of concurrent versus consecutive chemoendocrine therapy.

A study was set up in the UK in 1993 to address the question of combined chemotherapy and endocrine therapy. The ABC (Adjuvant Breast Cancer) Trial aims to test whether adjuvant chemotherapy and/or ovarian suppression add to the benefits of tamoxifen. All patients receive tamoxifen and randomisation for postmenopausal women is for, or not for, chemotherapy. Pre and perimenopausal women are randomised into one of two groups, with an elective decision being made for the other treatment:

- Chemotherapy or no chemotherapy (elective ovarian suppression or not)
- Ovarian suppression versus no suppression (elective chemotherapy or not)

The trial opened in 1993 and has an accrual target of 8,000. As of June 1998 2,638 patients had been entered into the study (UKCCCR Register of Cancer Trials). A further trial is ongoing in Scotland for premenopausal women for whom clinicians feel that tamoxifen treatment on its own is inadequate. The Scottish Chemo-Endocrine Trial D is being undertaken in collaboration with the EORTC and all patients receive chemotherapy and are randomised to receive tamoxifen or not.

An adjuvant trial in post-menopausal breast cancer investigating treatment with a combination of tamoxifen and anastrozole (Arimidex®) is being co-ordinated by the CRC Breast Cancer Trials Group in collaboration with Zeneca. Anastrozole is an aromatase inhibitor and acts by decreasing the level of circulating oestrogen by inhibiting the production of oestradiol from oestriol and oestrone in the adrenal gland. Aromatase within the breast is also a significant source of oestrogen and therefore of oestrogen mediated tumour proliferation (Brodie et al., 1999). The primary objectives of the ATAC trial are to compare the equivalence of tamoxifen and anastrozole and to compare the difference between tamoxifen alone and the combination of tamoxifen and anastrozole as adjuvant therapy, in terms of time to recurrence and tolerability. The trial opened in July 1996 and recruitment stopped on 31st October 1999 with >9000 patients entered. Two studies are also ongoing in Europe in which young, premenopausal women are being treated with combination endocrine therapy comprising tamoxifen and the LH-RH antagonist, goserelin. Adjuvant chemotherapy is an elective therapy in one study and the comparative therapy in the other. Over 3,500 women have been randomised.

2.6 Primary systemic therapy

Neoadjuvant chemotherapy is well established in the treatment of large primary breast tumours (Smith et al., 1995). The rationale of this treatment is to reduce tumour bulk and down-stage the tumour so that it can be successfully treated with less radical surgical treatment. This methodology can also be applied to endocrine therapy in endocrine-responsive tumours. Thus, primary endocrine therapy can be used to assess the response of the tumour to systemic therapy which can then be administered in the adjuvant setting after local treatment. In Edinburgh, the policy since the mid-1980's has been to delay the local surgical treatment in women with large, but technically operable tumours (greater than 4 cm in maximum diameter) until the response to systemic therapy has been assessed. In a pilot study of 88 women,

tamoxifen was the primary therapy of choice in postmenopausal women with ER positive tumours (20 fmol/mg cytosol protein or above) (Anderson et al., 1991).

2.7 Breast cancer treatment in elderly patients

Tamoxifen has been used as primary treatment in elderly women not considered suitable for surgery. When oestrogen receptor content was used to select patients suitable for conservative tamoxifen treatment, early progressive disease was markedly reduced at 6 months, from 30% in the unselected control group to 2% in the study group ($p < 0.001$) (Low et al., 1992). However, although some women maintain a long-lasting complete remission, the use of tamoxifen alone as first-line therapy is associated with a high risk of treatment failure (Bergman et al., 1995).

More recently, controlled trials in elderly patients with operable breast cancer have demonstrated the importance of local cancer control and highlighted the increased relapse rate and mortality in patients receiving sub-optimal surgical treatment. Modern anaesthesia has reduced the risks of major therapy and the low rate of post-operative mortality and high rate of tumour control makes local surgery the preferred primary therapy for many elderly patients (Fentiman, 1997). Further, patients progressing on endocrine therapy or whose metastatic disease is life-threatening should be considered for chemotherapy. Older women in generally good health tolerate standard doses of chemotherapy as well as their younger counterparts (Muss, 1994).

Current thinking is therefore that patients should be treated according to their biological, rather than chronological age and tamoxifen therapy alone should only be used for elderly breast cancer patients who are too frail or unwilling to undergo surgery or radiotherapy.

2.8 Chemoprevention

The first evidence of a significant reduction in contralateral breast cancer in patients receiving tamoxifen therapy was published in the *Lancet* in 1985 (Cuzick & Baum, 1985). Further evidence accrued from the Stockholm adjuvant tamoxifen trial of a significant reduction in the incidence of contralateral breast cancers in the tamoxifen treated patients (RR 0.55, 95% CI:0.31 – 0.98) (Fornander et al., 1989). The 1990 EBCTCG overview of randomised clinical trials of adjuvant therapy for early stage breast cancer demonstrated a 39% odds reduction of developing cancer of the contralateral breast in those women receiving adjuvant tamoxifen (Early Breast Cancer Trialists' Collaborative Group, 1992). One large case-control study identified 234 cases of contralateral breast cancer through a population-based cancer registry. Controls were matched for age, stage of disease and year of initial breast cancer diagnosis. Tamoxifen therapy, of mean duration less than 2 years, was associated with a decreased risk of a second primary breast cancer (matched odds ratio=0.5, 95%CI: 0.8-0.9) (Cook, 1995).

These data, together with laboratory evidence that tamoxifen interferes with the initiation and promotion of mammary cancer (Jordan & Morrow, 1993), that its action is tumouristatic rather than tumouricidal (Jordan, 1988) and the lack of adverse side effects, suggested that tamoxifen may have a role in the chemoprevention of breast cancer. A number of studies have been initiated to investigate the use of tamoxifen in the chemoprevention of breast cancer. The Royal Marsden pilot chemoprevention study has reported a planned interim analysis which did not show any effect of tamoxifen on breast cancer incidence in healthy women with a family history of breast cancer. The analysis included 2471 women (1238 tamoxifen, 1233 placebo) with a median follow-up of 70 months and there were 34 cases of breast cancer in the tamoxifen arm and 36 cases in the placebo arm (Powles et al., 1998). A study started in 1992 in Italy accrued 5408 women. The median follow-up is 46 months and there have been 19 and 22 cases of breast cancer in the tamoxifen and

placebo arms respectively (Veronesi et al., 1998). The US BCP Trial, under the auspices of the NSABP, began recruiting patients in 1992 and news that it was to be stopped more than a year early was announced on April 6th 1998 (NCI, Press Release). Analysis showed a 49% reduction in breast cancer incidence among the high-risk patients who took tamoxifen. A total of 13,388 women, aged 35 and older, were recruited (6,681 in the tamoxifen arm and 6,707 in the placebo arm) with a mean follow-up of about 4 years. Of women who received tamoxifen, 84 (17.9 per 1,000 women) developed invasive breast cancer, compared with 154 (32 per 1,000 women) who received placebo (Fisher et al., 1998). The IBIS study is being co-ordinated in the UK and has been underway since November 1993. The trial aims to randomise 7,000 women, with a strong family history of breast cancer, to receive 20 mg tamoxifen daily for 5 years, or placebo. As of September 1998 a total of 4,750 patients have been recruited from the UK, Spain, Finland, Switzerland, Belgium, Australia and New Zealand.

A major issue arising from these trials is that, although most women will not develop breast cancer, half will potentially be at risk of encountering the symptoms and side effects of tamoxifen. The unblinding and early termination of the BCP Trial has stirred some controversy, with accusations that the data cannot contribute to the question of duration of tamoxifen treatment. Another fundamental, and as yet unanswered, question is whether the reduction in incidence seen in the study will translate into a comparable reduction in mortality. Meanwhile, the UK and Italian studies are continuing.

In addition to its potential use in breast cancer prevention, there is evidence to suggest that tamoxifen may have chemopreventative effects in other cancers. For example, a reduced (although not statistically significant) incidence of 1,2-dimethylhydrazine-induced colon cancers was observed in rats fed tamoxifen daily, compared with controls (Ziv et al., 1997).

2.9 Mechanisms of tamoxifen action

2.9.1 Inhibition of oestrogen receptor

Oestrogen causes replication of breast cancer cells by binding to high affinity protein binding sites located within the nucleus of the cell. This binding of oestrogen to oestrogen receptors activates a cascade of subcellular events. These include the induction of progesterone receptor, an increase in the stimulatory growth factors TGF α and IGFII and a decrease in the inhibitory factor TGF β . Cell growth requires the synergistic actions of growth factors in the correct sequence and combination and inhibition of one or more has a profound inhibitory effect on cell growth. An example of the effect of tamoxifen on these processes has been shown in the expression of insulin-like growth factors (IGFI and IGFII) and TGF α . For example, serum IGFI levels, determined by radioimmunoassay, were significantly lower in 19 tamoxifen patients after two years treatment, than in 19 controls ($p < 0.05$). The effect was greater in postmenopausal than in premenopausal women (Friedl et al., 1993). It has also been observed that tamoxifen lowers the production of TGF α in ER positive tumours (Noguchi et al., 1993). It has been difficult to demonstrate an effect of tamoxifen on TGF β production by ER positive breast cancer cell lines in vitro and in fact there are data suggesting that TGF β signaling does not play a role in the tamoxifen mediated growth inhibition of hormone dependent cell lines (Koli et al., 1997).

Early in vitro work supported the view that tamoxifen is a competitive inhibitor of oestrogen and acts by binding to the oestrogen receptor, thereby blocking the action of oestrogen (Jordan, 1976). Two experimental systems, the dimethylbenzanthracene (DMBA)-induced rat mammary carcinoma model and the immune deficient (athymic) mouse implanted with MCF7 breast cancer cell lines, provided much of the early information supporting the use of long-term adjuvant tamoxifen therapy as a chemosuppressive strategy in breast cancer (Jordan, 1988). In both models tumour growth is suppressed by tamoxifen but re-growth occurs when treatment is stopped,

indicating a cytostatic rather than cytotoxic action. However, care is required when extrapolating these in vitro results to the human situation because of the differential effect that tamoxifen exerts depending on the species and tissue being studied.

More recent research has further elucidated the molecular mechanisms involved in the binding to oestrogen receptor. Oestradiol binds to the oestrogen receptor which then activates, dimerises and binds to an oestrogen response element (ERE). This in turn activates gene regulation. There are therefore numerous opportunities for an anti-oestrogen to confound oestrogen action and it is thought that specific antioestrogenic action may differ depending on the compound being studied (Jordan, 1994). Tamoxifen and related triphenylethylene-type antioestrogens bind to the oestrogen receptor and cause a change in folding of the steroid binding domain which blocks oestrogenic action and prevents gene activation. The pure antioestrogens have a different mode of action and appear to cause a rapid loss of receptor allowing little opportunity to activate the ERE thus halting oestrogen-regulated gene function.

2.9.2 Hormone independent mechanisms

Growth factors

In the order of 10% of oestrogen negative tumours respond to tamoxifen treatment. This observation has led to a proliferation of research on the hormone-independent mechanisms by which tamoxifen may exert its effects. Such non ER mediated action requires higher concentrations of tamoxifen and is observed at concentrations above 10^{-6} M. The hormone independent anticancer effect may in part be mediated by direct interaction of the antioestrogen with the transcriptional activation of responsive genes and a consequent modulation of growth factors. For example, tamoxifen has been shown to increase TGF β production in cultured fibroblasts (Colletta et al., 1990) and increased levels of stromal TGF β have been demonstrated in biopsies of both ER negative and ER positive breast tumours, after 3 months of tamoxifen treatment (Butta et al., 1992).

Apoptosis and Cytotoxicity

The binding of tamoxifen to specific antioestrogen receptors and also to other non-specific sites raises the intracellular concentration of tamoxifen and its principal metabolite, N-desmethyltamoxifen. At these higher concentrations a non-specific cytotoxic effect of tamoxifen has been observed and a variety of mechanisms have been postulated as contributing to this effect. This hormone-independent cytotoxicity may, in part, be explained by the effect of antioestrogens on programmed cell death or apoptosis. This process is controlled by genes, such as c-myc, erb-B2, bcl-2, p53, which respond to intra and extracellular signals. Antioestrogens contribute to complex genomic effects which result in the removal of inhibitors to apoptosis and a cytotoxic effect, independent of hormone responsiveness of the tumour (Kellen, 1996).

A tamoxifen-induced increase in intracellular ionised calcium concentration and cell death has been demonstrated at tamoxifen concentrations above 10^{-6} M, which is the range at which non-ER mediated cytotoxicity is reported (Jain & Trump, 1997). Interaction of tamoxifen with calcium ions (Ca^{2+}) has been implicated by a significant correlation between the growth inhibitory potency of triphenylethylene derivatives in vivo and their ability to inhibit Ca^{2+} -calmodulin-dependent cAMP phosphodiesterase in vitro, which is involved in cell signalling. (Fanidi et al., 1991). Another potential interaction of tamoxifen with Ca^{2+} is suggested by the observation that tamoxifen suppresses the invasive phenotype of breast cancer cell lines and restores the function of the E-cadherin/catenin complex. Alterations of function or expression of this tumour suppressor complex are frequent features of invasive cells. This effect of tamoxifen is influenced by Ca^{2+} channel modulators and may depend on a Ca^{2+} pathway (Charlier et al., 1996).

Human breast tumours contain elevated levels of protein kinase C (PKC) which is inhibited by higher concentrations of tamoxifen in vitro. PKC in turn regulates the phospholipase D system which is thought to be an important component of cell signal

transduction. It has been shown that longer-term (24-hour) treatment with tamoxifen can inhibit phospholipase D activity in an ER negative subline of MCF-7 human breast cancer cells, and this action may contribute to the hormone-independent cytotoxic effects of tamoxifen (Kiss & Anderson, 1997).

A study has been reported in which mice received cytotoxic T lymphocytes isolated from other tumour bearing animals and then were treated with oral tamoxifen. An enhanced tumour suppressor response was seen in these animals. Tamoxifen seems to increase host resistance by sensitising the tumour target to killer cell mediated lysis (Nagy et al., 1997).

Angiogenesis

Tamoxifen has been shown to exert angiostatic activity which is not related to the inhibition of oestrogen action. It is suggested that this activity may contribute to the therapeutic effect of tamoxifen in ER negative tumours (Gagliardi & Collins, 1993). The inhibition of angiogenesis and subsequent endothelial growth causes reduced vascularization and impaired tumour perfusion with resultant enhanced necrosis and tumour regression. Magnetic resonance imaging in MCF7 human breast cancer cells, implanted in nude mice, has demonstrated a highly significant decrease in endothelial density and a significant increase in the extent of necrosis of tumours (Haran et al., 1994).

Reversal of Multidrug Resistance

Multidrug resistance (mdr) is a major obstacle to successful cancer chemotherapy. The most widely implicated mechanism is the P-glycoprotein efflux pump which is a product of the *mdr1* gene and results in a reduced concentration of the cytotoxic agent within the cell and a reduction in cell kill. Tamoxifen has been shown to bind to P-glycoprotein, thus inhibiting the transport of cytotoxic drugs out of the cell. The

cellular accumulation of tamoxifen is not affected and therefore it does not appear to be transported by the protein (Callaghan & Higgins, 1995).

2.9.3 Antioxidant actions of tamoxifen

Current evidence clearly indicates that free radicals play a prominent role in the incidence and progression of breast cancer (Malins et al., 1996, Murrell, 1991). Tamoxifen is an effective antioxidant and protects membranes and low density lipoprotein (LDL) particles against oxidative damage. The protection by tamoxifen of cellular membranes, including the nuclear membrane, against the formation of genotoxic reactive intermediates and products of lipid peroxidation, could be important in the prevention of nuclear DNA damage. Such an anticarcinogenic mechanism may be important in the use of tamoxifen in the prevention of breast cancer (Wiseman & Halliwell, 1994). The tamoxifen metabolite, 4-hydroxytamoxifen, is a more potent anticancer agent than the parent molecule and is also a more powerful inhibitor of lipid peroxidation. This enhanced activity is thought to be due to the ability of the hydroxyl group to donate a hydrogen atom to quench free radicals capable of initiating the membrane oxidative degradation (Custodio et al., 1994).

The primary mediator of tamoxifen's antioxidant action is thought to be the ability to decrease membrane fluidity and enhance membrane stability. Membrane stability is very important for the normal functioning of membrane proteins. A positive correlation has been demonstrated in ox-brain phospholipid liposomes between the decrease in membrane fluidity conferred by tamoxifen and related compounds, and their antioxidant ability as inhibitors of liposomal and microsomal lipid peroxidation (Wiseman et al., 1993).

The antioxidant effect of tamoxifen has been shown clinically with a significantly decreased rate of lipid peroxidation demonstrated in 64 postmenopausal women after 3 and 6 months tamoxifen treatment, compared with controls. Increased levels of

selenium and the antioxidant vitamins A, C and E were also detected in the tamoxifen treated women (Thangaraju et al., 1994).

As well as playing an important role in the anticancer action of tamoxifen and contributing to its observed cardioprotective effect (*Chapter 3*), membrane-mediated mechanisms, through a putative modulation of membrane fluidity, are also likely to play a role in the ability of tamoxifen to reverse multidrug resistance and could also lead to clinical uses as an anti *Candida* and antiviral agent (Wiseman, 1994).

2.10 Tamoxifen resistance

Resistance to tamoxifen therapy can be classified as ‘de novo’ or ‘acquired’. De novo, or intrinsic, resistance is shown in 65% of patients with assessable disease and 40% of these will be ER positive tumours. Only 15% of these non responders will respond to a second-line endocrine agent. Tumours which respond initially to first-line tamoxifen therapy will, invariably, acquire resistance to treatment and relapse. This is often seen as a tamoxifen withdrawal response with further clinical response occurring when treatment is stopped. This phenomenon suggests that tamoxifen stimulates tumour growth prior to cessation of treatment. Unlike de novo resistance, 50% of these who develop resistance will respond to second line treatment.

Mechanisms of acquired tamoxifen resistance have been studied in the laboratory using cell lines and tumours which have been developed to be capable of growth in the presence of clinically relevant concentrations of tamoxifen. Tissue culture has shown that mutations in the oestrogen receptor can cause a receptor occupied by an antioestrogen to behave as though it were occupied by oestrogen and display tamoxifen stimulated growth (Wolf & Jordan, 1993).

Resistance to tamoxifen therapy may develop due to changes at the level of the ER itself or at pre and post receptor points in the ER response pathway. Several

mechanisms have been proposed but a single, distinct mechanism has not been identified. There are a number of steps within the ER mediated signal transduction pathway which may be altered and lead to tamoxifen resistance. These include:

- defects in the ER – such as a defect in phosphorylation
- loss or mutation of the ER
- defects in ER post-translational modification
- alteration of the oestrogen response element (ERE)

These proposed mechanisms are discussed in detail elsewhere (Tonetti & Jordan, 1995, Lykkesfeldt, 1996).

Resistance may also be mediated by changes in the antioestrogen itself, including altered uptake, retention and metabolism. There are clinical data showing reduced levels of tamoxifen in the tumours of patients with acquired resistance. This suggests that reduced uptake or increased efflux from cells may be implicated (Johnston et al., 1993)

Antioestrogens working by a different mechanism of interaction with the ER should prove useful in the treatment of some tamoxifen resistant breast cancer (Katzenellenbogen et al., 1997). The new pure antioestrogens appear not to be affected by mutations in the ER and can control tamoxifen stimulated growth. Such compounds may prevent the development of receptor mutants if used as first-line therapy (Jiang & Jordan, 1992).

3.1 Cardiac effects

3.1.1 Clinical studies

Data showing that tamoxifen therapy reduces the incidence of cardiac events are accumulating. Two studies looking at cardiac morbidity and mortality in patients entered into the Scottish adjuvant tamoxifen trial have previously been published (*Appendices 1 and 2*). The first of these demonstrated a significant reduction in the incidence of fatal myocardial infarction (MI) in patients randomised to tamoxifen (10) compared with the control group in (25) ($p=0.0087$). Only patients who were free of distant disease at the time of death were included in the analysis, thereby removing the confounding effects of metastatic disease and its treatment on the results. Information for analysis was obtained from the review of patients' hospital case-notes. The second study considered non fatal as well as fatal outcomes by looking at hospital admissions, within the trial population, which were identified on the hospital discharge summary as being due to cardiovascular disease. This study made use of the ability to link external databases to the computerised record of the hospital discharge. Use or intended use of tamoxifen was associated with lower rates of hospital admission for MI. The hazard ratio for women in the control group was 1.92 (95% CI: 0.99 to 3.73) compared with women allocated to adjuvant treatment. The association was stronger for current use with a hazard ratio for non users of 3.49 (95% CI: 1.52-8.03, $p=0.001$) compared with current users (McDonald et al., 1995).

A facility similar to that in Scotland which allows external databases to be linked to hospital records, exists in Sweden and one report (Rutqvist & Mattson, 1993) shows a significant reduction in hospital admissions for any cardiac disease in 2,365 postmenopausal patients randomised to tamoxifen use within the Stockholm adjuvant tamoxifen trial (relative hazard 0.68, 95% CI: 0.48-0.97). Median follow-up was 6 years. The study involved randomisation for adjuvant tamoxifen (40 mg per day for 2 years) or no adjuvant treatment. A further randomisation for those free from disease recurrence and still receiving tamoxifen at 2 years was to stop tamoxifen or continue

treatment for a further 3 years. A significant reduction in cardiac morbidity was also seen in those receiving tamoxifen for 5 years compared with those receiving treatment for 2 years (relative hazard 0.37, 95% CI: 0.15-0.92). In this study there was no significant reduction in women randomised to tamoxifen use in the hospital admissions for the subgroup suffering myocardial infarction.

These analyses were based on data collected in a computerised, population-based register of hospital admissions and corresponding discharge diagnoses, covering about 95% of all hospital admissions in the Stockholm area. The study differs from the Scottish study in that only an analysis of treatment allocated by randomisation was performed - the trial database did not contain sufficient information on tamoxifen use to allow analyses by actual tamoxifen status.

Data on mortality from coronary heart disease in patients with early stage breast cancer enrolled in the NSABP B-14 trial of tamoxifen therapy have been published (Constantino et al., 1997). The findings are consistent with the Scottish and Swedish results, with a reduction in death from MI, or other definite coronary heart disease, in patients randomised to 5 years of adjuvant tamoxifen compared with those randomised to placebo. The data fail to reach statistical significance (relative hazard 0.66, 95% CI: 0.27-1.61), but the consistency of these results with those from the Scottish and Swedish trials confirm the need to continue follow-up of these studies to accumulate enough data so that reliable conclusions can be reached.

3.1.2 Mechanisms of action

Lipid profile

The protective effect of tamoxifen on the cardiovascular system is likely to be mediated, in part, by a reduction in low density lipoprotein (LDL), an increase in subclass 2 high density lipoprotein (HDL) and a resultant beneficial change in lipid profile. Studies in the 1950's demonstrated that the decrease of endogenous oestrogen secretion after the menopause is followed by an increase in serum lipid levels and a corresponding increase in coronary artery disease (Oliver, 1959). Premature cessation of ovarian function was shown to be associated with an increased risk of non fatal myocardial infarction (Rosenberg et al., 1981) . However, most published studies of lipids, lipoproteins and cardiovascular disease have been in men and in an overview of blood cholesterol levels in adults, concentrations of plasma cholesterol and its LDL fraction were cited as being related to coronary artery disease in men (The Expert Panel, 1988). A review suggests that cholesterol levels may not be as closely linked with coronary heart disease in women (Isles, 1993).

Nevertheless, if we accept that atherosclerotic cardiovascular disease is associated with high levels of plasma low density lipoproteins and low levels of circulating HDL cholesterol concentrations, then the reversal of these abnormalities should reduce cardiovascular disease. A review of 23 studies supports the hypothesis that the favourable effect of postmenopausal oestrogen therapy on the lipid profile results in a reduction of cardiovascular disease in women treated with oestrogen replacement therapy (Knopp, 1988). A reduced hospitalisation rate for active myocardial infarction has been observed in oestrogen users compared with non users (Henderson, 1988). Recent data has confirmed that current use of oestrogen replacement therapy is associated with decreased cardiovascular morbidity and a reduction in sudden cardiac death (Sourander et al., 1998).

Tamoxifen has been shown to exert a similar effect on lipid profile as oestrogen, both in animal studies and in humans. Studies in female Wistar rats with experimental atherosclerosis showed an increase in total plasma lipids in animals suffering from atherosclerosis. HDL cholesterol was increased and LDL cholesterol decreased in tamoxifen-treated groups (Vinitha et al., 1997). Concomitant rises in marker enzyme levels, such as serum transaminases, lactate dehydrogenase and creatine phosphokinase, were seen in the atherosclerotic animals and these effects were reversed in tamoxifen-treated groups (Vinitha & Sachdanandam, 1997). Similar changes in lipid profile have been demonstrated in other animal models, for example monkeys (Williams et al., 1997) and particularly in rats, with the ovariectomised rat model being characterised for studies in this area (Lundeen et al., 1997).

Work in transgenic mice has studied the effect of tamoxifen on increasing apolipoprotein (a) concentration, TGF β activity and lipid lesion development, which represents a major risk factor for vascular diseases including atherosclerosis, restenosis and stroke. Tamoxifen appears to break the feedback loop whereby increased apo (a) concentration causes inhibition of TGF β and further apo (a) increases (Bruning et al., 1988). Oral tamoxifen can prevent apo (a) accumulation and a sevenfold decrease in total cholesterol levels in mice has been demonstrated, irrespective of diet (Reckless et al., 1997).

A number of clinical studies have looked at the effect of tamoxifen on various blood parameters which are of relevance in cardiovascular disease. As part of a controlled trial of the use of tamoxifen in the treatment of mastalgia, a panel of haemastatic variables were measured. There was a reduction in LDL cholesterol and an increase in subclass 2 of HDL cholesterol. No alteration was seen in clotting function but hepatic function was altered, with an increase in concentration of sex hormone binding globulin (Caleffi, 1988). A randomised, double blind clinical study of two years of tamoxifen versus placebo, in women with axillary node negative breast

cancer, showed generally favourable effects on lipid and lipoprotein profile, with a decrease in total cholesterol and low density lipoprotein levels, in the tamoxifen treated women (Love et al., 1991). An analysis of lipid levels in 68 women randomised within the Italian trial of tamoxifen in the prevention of breast cancer has shown a significant reduction in total and LDL cholesterol levels in women treated with tamoxifen, over a 6 month treatment period (Mannucci et al., 1996).

A subset of patients in the Scottish trial showed a decrease in total cholesterol while on tamoxifen treatment but this effect was no longer apparent when treatment was stopped (Dewar et al., 1992). A decrease in total serum cholesterol, a decrease in LDL cholesterol and an increase in HDL2 cholesterol has been observed in breast cancer patients on tamoxifen therapy (Morales et al., 1996). Some changes are observed after as little as 3 months of tamoxifen therapy (Ingram, 1990) and are maintained after 6 months and 12 months of treatment (Love et al., 1990). The Royal Marsden pilot tamoxifen chemoprevention study has shown a significant reduction in serum cholesterol in tamoxifen-treated women which has been maintained out to 5 years (Powles et al. 1994). A rise in plasma levels of sex hormone binding globulin is indicative of the intrinsic oestrogenic action of tamoxifen in the liver (Bruning et al., 1988).

Arterial status

Another hypothesis proposed to explain the relationship between oestrogens and cardiovascular disease considers the effect of oestrogen on arterial flow. Oestrogen increases arterial flow with a 50% reduction in the impedance to flow within the artery. This effect is possibly mediated by a receptor mechanism in the muscularis of major vessels (Bourne et al., 1990). However, the same effect has not been seen with tamoxifen. In a randomised study looking at the pulsatility index of cerebral arteries in

postmenopausal women, oestrogen caused a significant reduction in the pulsatility index but tamoxifen had no effect. (Penotti, 1998).

Antioxidant properties

Much work has been done on the action of tamoxifen as an antioxidant and its ability to protect membranes against the damaging effects of lipid peroxidation (*Chapter 2, Section 2.9.3*). Oxidative damage to LDL plays an important role in the development of atherosclerosis (Wiseman, 1994, Steinberg et al., 1989). Tamoxifen has been shown to protect rat cardiac membranes against the damage caused by lipid peroxidation (Wiseman et al., 1993) and it can also protect human LDL against Cu^{2+} -dependent lipid peroxidation (Wiseman, et al., 1993). This antioxidant action is mediated by modulation of membrane fluidity and is in addition to the favourable effect of tamoxifen on lipid profile which has already been discussed.

3.2 Thromboembolic effects

3.2.1 Introduction

Early concerns about possible complications of tamoxifen treatment resulting from its oestrogenic actions focused on its effect on blood coagulation, and in particular on the activity of antithrombin III, the most important physiological inhibitor of blood coagulation. A number of case reports appeared in the literature, describing thromboembolic complications in patients receiving tamoxifen therapy, including a report on 7 patients who developed venous thrombosis or pulmonary embolism within 6 months of starting treatment (Lipton et al., 1984 (Dahan, 1985). Another case describes occlusion of the finger arteries and thrombotic retinal angiopathy during adjuvant tamoxifen treatment (Schlich et al., 1997).

However, thromboembolic disease is a recognised and relatively common cause of morbidity and mortality in cancer patients undergoing chemotherapy (Shlebak & Smith, 1997) and it can be difficult to disentangle these effects from the incidence of events precipitated by tamoxifen use in such anecdotal reports. The aetiology of thromboembolism in cancer patients is multifactorial and includes the release of procoagulants by tumour cells and effects of anticancer drugs on protein C, fibrinopeptide production and endothelial cell reactivity. There is reliable information available on the incidence of thromboembolism for patients with breast cancer (Levine, 1997). In patients with stage II disease receiving chemotherapy, the incidence of thrombotic complications is of the order of 5%. The risk in metastatic disease is likely to be greater, because of the increased tumour burden.

3.2.2 Antithrombin III activity

In 1984, a study in women with metastatic breast cancer demonstrated a reduction in functional activity of antithrombin III in 42% of patients receiving tamoxifen, compared with 9% in women not receiving tamoxifen (Enck & Rios, 1984). Other studies have demonstrated a depression of antithrombin III levels associated with tamoxifen use, but these levels were still within the normal range and not considered to be of clinical significance (Jordan et al., 1987) (Love et al., 1992) (Auger & Mackie, 1988) (Bertelli et al., 1988). These results may reflect an action of disease activity, rather than tamoxifen, on antithrombin III levels. The Royal Marsden pilot chemoprevention study has noted a decrease in both antithrombin III and fibrinogen levels, with no resultant detrimental effect on the ratio of these clotting factors, this being the important clinical parameter (Powles et al., 1994).

3.2.3 Clinical studies

Early results from the Royal Marsden pilot study of the use of tamoxifen in the chemoprevention of breast cancer showed no evidence of an increase in thromboembolic events after a maximum follow-up of 36 months (Jones, 1992).

However, the study of cardiovascular morbidity within the Scottish tamoxifen trial reported significantly more hospital admissions for the thromboembolic events of pulmonary embolism and deep vein thrombosis in current users of tamoxifen compared with those who were not current users (HR 0.4, 95% CI: 0.18-0.90, $p=0.025$) (McDonald et al., 1995) (*Appendix 2*). The comparison of the randomised groups for incidence of thromboembolic disease in the Scottish study was not significant and the Swedish study of tamoxifen morbidity, described in *Section 3.1.1*, also failed to demonstrate a statistically significant difference between the randomised groups in terms of first admission to hospital for thromboembolic disease (Rutqvist & Mattson, 1993).

The incidence of thromboembolism has been investigated in large randomised trials of combined chemotherapy and tamoxifen, given as adjuvant therapy. The results show a greater risk for patients receiving a combination of chemotherapy and tamoxifen than for those receiving either chemotherapy or tamoxifen alone. In a randomised study of tamoxifen versus tamoxifen plus 6 months of concurrent CMF chemotherapy, there were five (5) thromboembolic events in 352 women (2.6%) in the tamoxifen alone group and 48 in 353 women allocated to receive tamoxifen plus CMF (13.6%) (Pritchard et al., 1996). In a retrospective review of seven studies of adjuvant therapy (chemotherapy and/or tamoxifen) for breast cancer, the records of 2,673 women were reviewed for the occurrence of vascular complications. For premenopausal patients, the frequency of thromboembolic events was 0.8% in patients receiving chemotherapy and 2.8% in patients receiving combined chemotherapy and tamoxifen ($p=0.03$). A similar picture emerged in postmenopausal patients with an incidence of 2.3% and 8.0% in the tamoxifen and tamoxifen plus chemotherapy groups respectively (Saphner et al., 1991).

3.3 Carcinogenic effects

3.3.1 Introduction

Although a striking feature of the use of tamoxifen in the treatment of breast cancer is its low toxicity profile, one aspect of major concern is its carcinogenic potential. The clinical data, relating to the incidence of second primary tumours associated with breast cancer use, are ambiguous, due to inadequate surveillance and the difficulties in obtaining the histological diagnoses necessary to distinguish new primary tumours from metastatic relapse.

3.3.2 Mechanisms

It is postulated that free radical mechanisms may have a role to play in the carcinogenicity of tamoxifen. Although the molecule acts as an antioxidant towards lipids, it may exert pro-oxidant effects on other molecules, such as DNA (Wiseman & Halliwell, 1993). The major metabolite of tamoxifen, 4-hydroxytamoxifen, has been shown to be more rapidly susceptible to free radical breakdown, with a consequent potential to inflict greater DNA damage than tamoxifen itself (Davies et al., 1997). It has also been suggested that the carcinogenic effects seen with high doses of tamoxifen may be related to changes in drug metabolising and antioxidant enzyme activities induced by tamoxifen treatment (Ahotupa et al., 1994). In vitro studies, demonstrating the agonist activity of tamoxifen in the presence of mutated oestrogen receptors, suggest that mutations in the oestrogen receptor may account for the tamoxifen stimulated tumours seen clinically. However, this has not been confirmed in reproducible laboratory models of human breast and endometrial carcinomas (Bilimoria et al., 1996).

3.3.3 Hepatocellular carcinogenicity

Animal Studies

Animal studies have confirmed the ability of tamoxifen to induce liver cancer in rats, this effect being dose, duration and species dependent (Greaves et al., 1993). The doses given in the study were high (5, 20 and 35 mg/kg per day) and an increase in the incidence of hepatocellular carcinomas was observed after 31 weeks of treatment in the highest dose group. At doses of < 3 mg/kg per day, no hepatoproliferative changes were found in rats exposed for up to one year (Williams et al., 1993). The dose-response curve is very steep; no carcinogenic effect was seen at doses below 3 mg/kg whereas concentrations of 15 mg/kg produced liver tumours in 50% of animals, after treatment for one half of a lifetime (Jordan & Morrow, 1994). Studies using a more realistic model, administering the therapeutic dose (~0.25 mg/kg) and equivalent times and durations to those used clinically, have not been undertaken, but these dose regimens are below the level that causes adduct formation, suggesting that it is unlikely that the animals would develop liver tumours, as is the clinical experience. However, in some strains of rat, tamoxifen can cause cancers in the liver at blood levels similar to those in women being treated for breast cancer with this agent (Robinson et al., 1991). In contrast to the effect in rats, tamoxifen is non carcinogenic in mice because it does not cause sufficient cumulative DNA damage, or act as a promoter by causing cell proliferation (Martin et al., 1997). Hepatocellular carcinogenicity has not been reported in any other animal species.

Clinical Data

Fears of the results seen in rats being reproduced in humans have been allayed to some extent. Early data from Sweden reported two cases of hepatocellular carcinoma in the adjuvant group compared with none in the control group (Fornander et al., 1989). However, no subsequent study has reported an excess of liver cancers associated with tamoxifen use. There are two possible explanations for the difference

between the preclinical data and clinical experience. Firstly, the dose and duration of treatment in the human is a fraction of that used to initiate carcinogenesis in the rat. Secondly, a study looking at the number of adducts in rats and humans found the number of adducts in rats were two orders of magnitude greater than the number in humans. In humans the number of adducts in the tamoxifen treated group was not significantly different from the control group (Martin et al., 1995).

A combined analysis of the results from the Stockholm Breast Cancer Study Group, the Danish Breast Cancer Group Trial and the South-Swedish Trial found no evidence that tamoxifen increases the incidence of liver cancer in humans (Rutqvist et al., 1995). This is in spite of the high daily dose given (40 mg daily), which is twice that used in the UK and the US.

DNA Adducts

The hepatocarcinogenic effects of tamoxifen are attributed to the formation of DNA adducts in the liver, in a time and dose-dependent manner. Activation of tamoxifen by cytochrome P450 drug-metabolising enzymes results in reactive intermediates that bind covalently to DNA to form adducts. It has been postulated that conjugation of alpha-hydroxytamoxifen takes place in the liver to give an ester which alkylates DNA (Osborne et al., 1997). Other mechanisms have also been proposed by which a reactive tamoxifen intermediate, capable of forming DNA adducts, is produced by epoxidation reactions of tamoxifen (Tonetti & Jordan, 1996).

In vitro experimental systems have shown that tamoxifen derived DNA adducts, alpha-(N-2-deoxyguanosinyl) tamoxifens, have high miscoding potentials (Shibutani & Dasaradhi, 1997). Local detoxifying mechanisms and DNA repair will limit the likely expression of clinical cancer. At present, there are few intermediate markers of carcinogenic risk and quantitative prediction of any human risk is not possible. There are few data relating to levels of DNA adducts in the liver in tamoxifen treated

patients. One small clinical trial found no difference in the level of adducts in women being treated with tamoxifen compared with controls (Martin et al., 1995). More recently, increased levels of adducts have been detected in leukocyte DNA from eleven breast cancer patients. The level of adducts in 6 tamoxifen-treated patients was 5.5 adducts/109 nucleotides as compared with a level of 1.9 adducts/109 nucleotides in 5 untreated controls (Hemminki et al., 1997).

3.3.4 Uterine carcinogenicity

The true incidence of endometrial cancer, related to the oestrogenic effect of tamoxifen, is difficult to estimate from case reports because breast and endometrial cancer share hyperoestrogenism as a risk factor. Body fat distribution is an important variable in relation to endogenous oestrogen production and women with excess upper body fat may have an inherent risk of developing endometrial lesions, even when not taking tamoxifen.

Since the demonstration in 1988 of the ability of tamoxifen to stimulate the growth of a hormone responsive endometrial cancer and block the oestrogen dependent growth of a breast cancer co-transplanted in the same athymic mouse (Gottardis et al., 1988), there has been concern about the ability of tamoxifen to cause endometrial cancer in women. Hardell suggested a causal relation between tamoxifen and the risk of uterine cancer in 1988 (Hardell, 1988).

Tamoxifen's oestrogen-like action on the uterus may combine synergistically with its known genotoxic effect to make the uterus a potential prime site for carcinogenic risk in humans. In a study of 19 postmenopausal, tamoxifen treated breast cancer patients who underwent endometrial sampling, tamoxifen associated changes in endometrial steroid receptors were identified, supporting an oestrogenic effect which may contribute to the pathogenesis of polyps and carcinomas associated with tamoxifen use (Schwartz, 1997). In vitro studies using endometrial cell lines, have demonstrated

oestrogen agonist effects of tamoxifen on proliferation and plasminogen activation (Hochner-Celnikier et al., 1997). The effect of tamoxifen on the endometrium is related to cumulative dose and duration of exposure. This dose dependency is supported by a prospective study of 19 tamoxifen treated breast cancer patients which identified a spectrum of pathological findings, with endometrial malignancies confined to those who had taken more than 35 g of tamoxifen (Ismail, 1995).

The evidence of an association between tamoxifen and endometrial carcinoma is based primarily upon clinical observation rather than laboratory data. Early clinical evidence of an increased incidence of endometrial cancer associated with tamoxifen use was seen in a case report in 1985 (Killackey et al., 1985). By 1995, there were reports of more than 250 cases of endometrial cancer in women taking, or having completed, tamoxifen therapy (Assikis & Jordan, 1995).

Observations in randomised clinical trials of adjuvant tamoxifen therapy are consistent with a small, but real increased risk of endometrial cancer in women who take tamoxifen. These data are tabulated in *Table 3.1*. A review of the literature on the carcinogenic potential of tamoxifen was published in 1998 and concludes that the data suggest that tamoxifen may be a tumour promoter in the human endometrium (Stearns & Gelmann, 1998). There are problems in obtaining data on the risk of endometrial cancer in studies which were not designed to assess this outcome. For example, information on risk factors, such as exogenous oestrogen exposure, is not collected. Although patients in randomised studies undergo more extensive medical evaluation with a possibility of detection bias, this should not differ in tamoxifen and control groups.

Table 3.1

Trial	Dose g/day	Duration	No. of patients	RR	95% CI
Stockholm ¹	40	2-5 yrs	2729	5.6	1.9-16.2
Danish Breast Cancer Group ²	30	48 weeks	1710	3.3	0.6-32.4
South Sweden ³	30	1 year	719	NS	NS
Combined Scandinavian * ¹	As above	As above	4914	4.1	1.9-8.9
NSABP ⁴	20	5 years	2843	7.5	1.7-32.7

Relative risk of endometrial cancer in some large adjuvant tamoxifen trials

*Combined analysis of the Stockholm, Danish Breast Cancer Group and South Sweden trials

¹ (Rutqvist et al., 1995)

² (Andersson et al., 1992)

³ (Ryden et al., 1992)

⁴ (Fisher et al., 1994)

One of the earliest randomised trials of adjuvant tamoxifen was conducted by the Novaldex Adjuvant Trial Organisation (NATO). Between 1977 and 1981 1,285 patients were randomised to receive adjuvant tamoxifen, 20 mg per day for two years, or no further therapy. After 6 years of follow-up no cases of endometrial cancer had been reported (Novaldex Adjuvant Trial Organisation, 1988). Several other randomised studies, some involving only one year of tamoxifen therapy, have also looked at the incidence of endometrial cancer and have shown no evidence of an increased risk (Stewart & Knight, 1989, (Ribeiro & Swindell, 1992, Hubay et al., 1985, Castiglione et al., 1990, Boccardo et al., 1992, Cummings et al., 1993). The Royal Marsden pilot tamoxifen chemoprevention trial reported two cases of endometrial cancer, both in the tamoxifen arm in which women received 20 mg tamoxifen per day for up to 8 years (Powles et al., 1994).

There have also been case-control and cohort studies reported in the literature. One case-control study identified 98 patients who had endometrial cancer diagnosed at least 3 months after a primary breast cancer, through the population based Netherlands Cancer Registry and two hospital based registeries. Controls (285) were matched for age, year of breast cancer diagnosis and survival time with an intact uterus. Tamoxifen had been used by 24% of patients with subsequent endometrial cancer and 20% of controls (RR 1.3, 95%CI: 0.7-2.4). The relative risk rose to 2.3 (95% CI: 0.9-5.9) in women who had used tamoxifen for more than two years. There was a significant trend of increasing risk with duration ($p=0.049$) and cumulative dose ($p=0.046$). In this study, the duration-response trends were similar with daily doses of 40 mg or 30 mg and less (van Leeuwen et al., 1994).

A nested case-control study identified 42 cases of endometrial cancer following a primary breast cancer. Controls (two controls for every case) were matched to cases on age, year of initial breast cancer diagnosis and disease stage. The mean duration of tamoxifen use was less than 2 years and the results were consistent with no

association (matched odds ratio 0.6, 95%CI: 0.2-1.9) This study also identified 39 cases of second primary ovarian cancers and showed no association of development of this tumour with tamoxifen use (matched odds ratio 0.6, 95% CI: 0.2-1.8) (Cook, 1995).

A case-control study involving 1,017 patients treated at one centre for primary breast cancer between 1978 and 1989 was undertaken. Four hundred and thirty one (431) cases were excluded because of inaccurate records (56) or a previous hysterectomy (375). Information on confounding variables was collected and exposure to tamoxifen was found to be an independent risk factor for the development of endometrial cancer (odds ratio 15.2, 95% CI: 2.8-84.4) (Robinson et al., 1995).

The only published cohort study, in which the incidence in a group of subjects is compared to incidence in the general population as a factor of exposure to the suspected causal agent, is the incidence of second primary cancers among 87,323 women with breast cancer reported to the SEER (Surveillance, Epidemiology and End Results) Program. A significant excess of uterine corpus cancers was seen in the tamoxifen treated group (Ratio of observed to expected 2.03, 95% CI: 1.59-2.55) (Curtis et al., 1996).

Grade of endometrial cancer

In 1993, a retrospective study looking at the histological features of uterine cancer in 53 patients with a history of breast cancer was published. Fifteen of the subjects had received tamoxifen as adjuvant therapy. The results suggested that tamoxifen is associated with high-grade endometrial cancers having a poor prognosis (Magriples et al., 1993). This led to speculation that tamoxifen may be causing progression of pre-existing disease.

A retrospective review of clinical history and uterine pathology of 72 patients who developed malignant neoplasm of the uterine corpus after being treated for breast cancer with either tamoxifen or other therapeutic regimens revealed several features of the uterine malignancies. There was a previously unreported high incidence of clear cell carcinomas and leiomyosarcomas and a higher incidence of serous carcinoma in patients treated for 12 months or more (Silva et al., 1994).

Carcinosarcoma of the female genital tract is a relatively rare but well described neoplasm which usually arises in elderly women. It most commonly originates within the uterus and accounts for 1.5% of tumours of this organ. It is a biphasic tumour composed of an admixture of carcinomatous and sarcomatous elements. It is also referred to as malignant mixed mesodermal tumour or malignant mixed Mullerian tumour. Uterine carcinosarcoma is a highly aggressive neoplasm which usually deeply invades the myometrium and commonly results in metastatic disease.

There have been suggestions that prolonged, unopposed oestrogenic stimulation with tamoxifen may be a possible etiologic factor in the development of uterine carcinosarcoma. This is based on data, mainly from case reports, of an excess of this type of uterine tumour in patients receiving long-term tamoxifen therapy (McLuggage et al. 1997) (Altaras et al., 1993). If such an association did exist, this would be highly important given the aggressiveness of this lesion. However, the main body of evidence to date does not support the association of tamoxifen therapy with high-grade tumours, with the majority of uterine tumours reported in tamoxifen treated patients being of grade 1 and 2 endometrial carcinomas (Assikis & Jordan, 1995).

3.3.5 Other primary cancers

Although most interest on the carcinogenic effects of tamoxifen has focussed on liver and uterine cancers, some published data suggest an increased incidence of other primary tumours, associated with tamoxifen use. In the South Sweden adjuvant trial, a higher rate of new primary cancers, other than breast, in the tamoxifen-treated patients was reported (Ryden et al., 1992). In the Danish trial, involving 3,538 postmenopausal patients, data suggested tamoxifen related increases in gastrointestinal cancers, particularly colorectal, in patients at high risk of recurrence who had received radiotherapy (Andersson et al., 1991). A combined analysis of data from these studies, plus the Stockholm trial, was published in 1995, involving a total of 4,914 patients with a median follow-up of 8-9 years. The study reported an excess of gastrointestinal cancers in tamoxifen treated patients, due to an increase in colorectal cancers (RR 1.9, 95% CI:1.1-3.3) and stomach cancers (RR 3.2, 95% CI:0.9-11.7). These data suggest that gastrointestinal organs may be target sites for tamoxifen induced carcinogenesis in humans (Rutqvist et al., 1995). However, these results have not so far been supported by other studies (Curtis et al., 1996) (Fisher et al., 1996) and some doubt has been placed on the reliability of the data and the analytical methods used (Jordan, 1995) (Simon, 1995). A recent analysis of 793 colorectal cancers identified within the SEER programme indicated that overall there was no association between hormone therapy and colorectal cancer (RR 1.09, 95% CI: 0.88-1.35). However there was a suggestion of an increased risk after 5 years of treatment (RR 1.47, 95% CI: 1.00-2.15) (Newcomb et al., 1999).

A report was published in 1997 of two cases of acute myeloid leukaemia which developed during tamoxifen therapy for breast cancer (Yalcin et al., 1997). A significant increase in the incidence of non lymphatic leukaemia was observed in the Danish adjuvant tamoxifen trial. However, this was confined to those patients who had received postoperative radiotherapy, with or without adjuvant tamoxifen. (Andersson et al., 1991).

3.4 Other effects on the endometrium

A report in 1989 highlighted an increase in endometrial and endocervical polyps detected at hysteroscopy in patients treated with tamoxifen, together with evidence of a hyperplastic endometrial mucosa (Neven et al., 1989). Further evidence was obtained from the Royal Marsden pilot tamoxifen chemoprevention trial in which a randomised cohort of 111 postmenopausal women were studied by transvaginal ultrasonography, with colour doppler imaging and microscopic examination of endometrial biopsies. The study found evidence of a larger uterus in the tamoxifen group and a greater incidence of endometrial hyperplasia and polyps (Kedar, 1994).

A study in 175 postmenopausal breast cancer patients has demonstrated different coexisting histological features in the endometria of patients on tamoxifen treatment - atrophic endometrium frequently coexisted with simple or complex hyperplasia. The authors hypothesise that different endometrial sites may respond differently to tamoxifen (Cohen et al., 1997). A study has correlated endometrial thickness with duration of tamoxifen use greater than 5 years, in 91 postmenopausal breast cancer patients (Hann et al., 1997). Endometrial biopsy in women with endometrial thickness of 8 mm or more revealed a variety of pathologies including endometrial polyps, endocervical polyps and hyperplasia, more frequently than in biopsies of endometria less than 8 mm. There are also case reports of endometrial polyps induced by tamoxifen treatment (Eagle, 1996).

3.5 Skeletal effects

3.5.1 Introduction

Bone structure and metabolism

The skeleton carries out a range of mechanical functions ranging from transmission of sound waves in the middle ear to withstanding loads of many times body weight. In addition, bone plays a role in the metabolism of calcium and magnesium and is the major reserve of these elements. The mechanical and biochemical functions of bone are dependent on each other.

Overall, the adult skeleton comprises about 80% cortical bone and 20% trabecular (cancellous bone). The cylindrical shafts of the long bones, such as the radius, ulna and femur, are composed predominately of cortical bone. The expanded ends of long bones are composed of trabecular bone, as are the vertebrae. The amount of bone in the skeleton depends upon the balance of bone formation by osteoblasts and bone resorption by osteoclasts. The relationship between the two is dependent on age, with peak bone mass remaining relatively stable from the age of about 25 years until about 50 years. Uncoupling of the action of osteoblasts and osteoclasts leads to an increase of resorption over formation, with a resultant reduction in bone mass and atrophy of the skeleton. At all ages, women have a lower bone mass than men.

Ionic exchange on the surface of bone crystal provides a physical basis for the continuous process of remodeling and replacement of bone. About 700 mg of calcium is exchanged between blood and bone each day, and this process is assisted by the activity of bone cells and influenced by a number of factors. 1,25-dihydroxyvitamin D (calcitriol) and parathyroid hormone (PTH) both stimulate bone resorption, while calcitonin acts directly on osteoclasts to inhibit their activity. The secretion of PTH and calcitonin are controlled by calcium ion concentration in the extracellular fluid. A low concentration of calcium ion stimulates release of PTH, which mobilises bone calcium and increases bone resorption while a high calcium ion concentration

stimulates calcitonin release. At the molecular level, PTH is thought to act by inducing changes in cellular metabolism which alter the rate of nucleic acid and protein synthesis and thus the activity and number of bone osteoclasts.

3.5.2 Effects of oestrogen on the skeleton

Bone mass

After the menopause, when circulating oestradiol concentrations decrease, there is an acceleration of bone loss, which is particularly marked in trabecular bone. Typically, in early menopause, the magnitude of oestrogen related bone loss represents a two to threefold increase in turnover rate and results in a decrease in bone mineral density (BMD) of about 15% after 5 years (Marcus, 1997). Conjugated oestrogens were approved in the US for the treatment of menopausal symptoms in 1942, but it was not until 1986 that the FDA announced a group of oestrogen products which were effective in the treatment of postmenopausal osteoporosis. The clinical effects of oestrogen, with or without added progestin, were assessed on BMD of the spine and hip in a multicentre study conducted over 3 years. Unopposed oestrogen increased BMD by 5% at the lumbar spine and 2% at the proximal femur. Similar changes were seen with oestrogen plus medroxyprogesterone (PEPI Trial Investigators Writing Group, 1996).

Fractures

Postmenopausal bone loss is a major problem and an estimated 30% of all postmenopausal women have osteoporosis, according to a definition of bone mineral density being more than 2.5 standard deviations below the mean for young healthy adult women at any site (WHO Study Group on Assessment of Fracture Risk and its Application to Screening for Post Menopausal Osteoporosis, 1994). This reduction in bone mass brings with it an increased risk of fracture, particularly of the hip, vertebrae and distal radius. A meta analysis of prospective cohort studies published between 1985 and 1994, looked at the ability of a decrease in bone mineral density of



one SD below the age adjusted mean to predict fracture risk. Such a decrease measured at the spine equated with an increased risk of vertebral fracture (RR 2.3, 95% CI: 1.9-2.8) and an increased risk of hip fracture (RR 2.6, 95% CI: 2.0-3.5) (Marshall et al., 1996).

In spite of the recognised association between reduced bone mass and fracture risk and improved biochemical and densitometric methods of measurement, the use of measurements of bone mineral density to screen for fracture risk, at the time of the menopause, remain difficult in practice because there is no international consensus concerning the definition or threshold value for a high fracture risk (Ringa et al., 1994). Whether a low bone mass expresses itself as a fracture in the future depends on a number of factors; the occurrence of a fall and a lack of muscle and fat which may protect bone in the event of a fall. Most non-spine fractures result from a fall and recent case-control studies have shown that the mechanics of a fall are the most important determinant of whether it will result in a hip fracture (Cummings & Nevitt, 1994). It is suggested that it is important to determine not only the current status of bone mass but to develop methods to predict future bone loss. A combination of methods may be required to develop accurate predictive methods (McGowan, 1993). There is now evidence that genetic factors may play a role in the regulation of bone mass and that fracture risk may be due in part to changes in bone quality with a decrease in crosslinking resulting in increased fragility (Ralston, 1997).

Data on the efficacy of preventative interventions, such as hormone replacement therapy, on fracture incidence are relatively sparse compared with those showing effects on bone mass. A retrospective cohort study of 490 women (245 oestrogen users for at least 5 years, 245 age-matched postmenopausal non users) found a statistically significant reduction in the incidence of wrist and vertebral fractures in users compared with non users. The age-adjusted incidence ratio for wrist fractures was 0.55 (95% CI: 0.32-0.92) and for vertebral fractures was 0.57 (95% CI: 0.41-

0.80) (Maxim et al, 1995). In 1997 a review was published on the skeletal effects of hormone replacement therapy and considered aspects such as dose, duration and the age at which treatment should be initiated (Marcus, 1997).

Mechanisms

In vitro studies suggest that the action of oestrogen on bone involves a number of mechanisms. Studies of bone turnover in ovariectomized rats, using dynamic bone histomorphometry, showed that oestrogen prevents osteopenia, in part, by inhibiting bone turnover (Turner et al., 1993). In vitro studies of the effect of oestradiol (1 mmol/L) on osteoclast cultures from rat long bones, demonstrated a 25% inhibition of bone resorption (Tobias & Chambers, 1991). Oestrogen may also act, through functional oestrogen receptors present in bone derived cells, to increase bone formation and as well as inhibiting resorption by reducing responsiveness to PTH (Ernst et al., 1989). Avian osteoclasts contain high levels of oestrogen receptors and the inhibition of osteoclast resorption activity by oestradiol is thought to be mediated, at least in part, by the regulation of osteoclast lysosomal gene expression (Oursler et al., 1993). Oestrogen can also act directly on the thyroid C cell to stimulate calcitonin secretion and thus inhibit bone resorption (Greenberg et al., 1986), and there is evidence of the induction of apoptosis of the bone resorbing osteoclasts by an oestrogen receptor mediated mechanism (Kameda et al., 1997)

3.5.3 Effects of tamoxifen on the skeleton

The first suggestion that tamoxifen had an oestrogen like effect on bone, was the observation that breast cancer patients with bone metastases developed transient hypercalcaemia on tamoxifen therapy (Legha et al., 1981). Subsequently, studies in rats have demonstrated the oestrogenic action of tamoxifen in preventing bone loss. In male rats, tamoxifen was able to reduce the trabecular bone loss resulting from unilateral sciatic neurotomy, but had no effect on cortical bone disuse osteopenia or on trabecular bone formation (Wakley et al., 1988). Studies in ovariectomised female rats

have shown that ovariectomy results in large increases in the number and activity of osteoclasts. This results in increased bone resorption with a net loss of trabecular bone. Treatment with tamoxifen prevented these skeletal changes (Turner et al., 1988).

The effect of tamoxifen in preventing bone loss induced by ovariectomy has been shown to depend on the circulating oestradiol concentration. While tamoxifen has oestrogen agonist activity in oestrogen deficiency, it has antioestrogenic actions in the presence of oestrogen (Kalu et al., 1991). This effect was confirmed in later studies in which tamoxifen was shown to exert an oestrogen agonist effect in ovariectomised rats but produced a 31% bone loss in intact animals, demonstrating an oestrogen antagonistic effect (Sibonga et al., 1996), (Li et al., 1996). The interaction between tamoxifen and ovarian status observed in animal studies has implications for the use of tamoxifen in the treatment of pre and postmenopausal women, particularly in its use as a chemopreventative agent in younger women.

Mechanisms

The use of ovariectomised rats is a useful animal model of postmenopausal bone loss in which the characteristics of the bone loss and its sequelae resemble those found in postmenopausal women (Kalu, 1991). Ovariectomy causes an oestrogen deficiency, which results in an accelerated bone loss similar to that found in early menopause. This bone loss mimics postmenopausal bone loss in several ways;

- Increased rate of bone turnover, with resorption exceeding formation
- An initial rapid phase of bone loss is followed by much slower phase
- Greater loss of trabecular bone than cortical bone
- Obesity provides some protection to bone loss.

Similar skeletal responses to therapy with oestrogen, tamoxifen, bisphosphonates, parathyroid hormone, calcitonin and exercise have been observed in this model.

Most of the evidence relating to the effect of tamoxifen on the skeleton indicates an inhibition of bone resorption, rather than increased bone formation. A study in the ovariectomised rat model showed that oestrogen was able to restore spinal bone mineral density in rats with established osteopenia, whereas tamoxifen inhibited further bone loss but was unable to restore spine BMD (Li et al., 1998). A number of studies have tried to elucidate the mechanism of tamoxifen's action on the skeleton. In vitro studies have shown that tamoxifen can completely block PTH induced bone resorption at concentrations of 100 μ M. A lower concentration (10 μ M) of tamoxifen is ineffective at blocking resorption and concentrations of 40-50 μ M partially inhibited the response (Stewart & Stern, 1986).

Tamoxifen has been shown to have a strong, dose-dependent, inhibitory action on 1,25-dihydroxyvitamin D3 stimulated bone resorption in vitro (Vink van Wijngaarden et al., 1995). This result was in contrast to that of an earlier study in which rats were fed on low calcium diets to stimulate PTH secretion. Tamoxifen did not inhibit PTH or calcitrol mediated bone resorption (Goulding et al., 1990).

Clinical studies

In vitro and animal data showing an inhibitory effect on bone loss are supported, to a greater or lesser extent, by a number of clinical studies in which measurements of bone mineral density and bone turnover have been made. Early clinical studies with small numbers of women, having tamoxifen exposures up to two years, provided reassurance that tamoxifen was not exerting antioestrogenic effects on the skeleton and causing reductions in BMD (Love et al., 1988) (Turken et al., 1989). Lack of a detrimental effect on bone mineral density at any site was confirmed in a retrospective

study involving 19 tamoxifen treated patients and 19 age-matched controls. BMD was measured at the femoral neck, lumbar spine and total body by dual energy X-ray absorptiometry (Neal et al., 1993). Bone mineral density in women with breast cancer has been shown to be lower in postmenopausal women with breast cancer compared with controls, matched for age and number of years since menopause. Tamoxifen did not increase the age-related bone loss in these women (Kostoglou-Athanassiou et al., 1994).

In a two year randomised, double blind, placebo controlled trial of 140 women with early breast cancer, tamoxifen increased the mean bone mineral density of the lumbar spine compared with a decrease in those in the placebo arm. Tamoxifen had no effect on radial bone mineral density (Love et al., 1992). This differential effect of tamoxifen on cortical and trabecular bone was also seen in a double blind, placebo controlled trial involving 57 healthy postmenopausal women. In those randomised to tamoxifen, mean bone mineral density of the lumbar spine increased while a decrease was observed in the placebo group ($p < 0.05$). There was no significant effect of tamoxifen on bone mineral density in the proximal femur (Grey et al., 1995). Other studies have shown a stabilisation of bone mineral content in the forearms of tamoxifen treated patients compared with a decrease in controls (Kristensen et al., 1994). All of these studies demonstrated significant falls in biochemical markers of bone turnover, such as serum alkaline phosphatase and ionised calcium. There are published data suggesting that an exposure duration of as little as 10 weeks in women over 70 years of age, is effective in inhibiting bone turnover (Kenny et al., 1995).

Histological data on bone turnover, in 41 postmenopausal women, also provided early evidence that tamoxifen did not have an antioestrogenic effect on bone and indicated a possible oestrogenic effect. There was no evidence of an adverse or protective effect of long-term tamoxifen treatment of the elemental composition of bone minerals (Kalef et al., 1996).

Few data are available on the skeletal effects of tamoxifen therapy in premenopausal women. Evidence from the ovariectomised rat model suggests that, whereas tamoxifen mimics the action of oestrogen in the postmenopausal skeleton, it may have an antioestrogenic action on bone in the presence of circulating oestrogens (Sibonga et al., 1996). Short-term administration (3 or 6 months) of tamoxifen to premenopausal women with benign breast disease (mastalgia) did not measurably influence spinal or femoral bone density (Fentiman et al., 1989). However the interaction seen in the preclinical data is supported by data coming out of the chemoprevention studies, with a transient bone loss reported during the first two years of treatment in premenopausal women in the Royal Marsden pilot prevention trial (Powles, 1998). The effect of tamoxifen on the skeleton is therefore far from clear and may differ depending on menstrual status.

Fractures

Although there are a lot of published data relating to measurement of BMD in tamoxifen treated patients, there are little data relating these observed effects of tamoxifen on bone mineral density to the incidence of fractures in women who are receiving, or have received, tamoxifen treatment. To date, only one such study has been published by the Danish Breast Cancer Cooperative Group, in which the incidence of fracture in 1,716 postmenopausal women randomised into a trial of adjuvant tamoxifen was determined by data linkage with the Danish National Registry of Patients. The results show a similar incidence of fracture of the femur between the

tamoxifen and control groups (64 and 51 cases respectively). Eleven patients in the control group had one trochanteric fracture compared to 27 patients in the tamoxifen group (HR=2.12, 95% CI:1.12-4.01) suggesting that tamoxifen use may increase the risk of fractures at certain sites. (Kristensen et al., 1996).

3.6 Ocular effects

3.6.1 Mechanisms

The National Registry of Drug-Induced Ocular Side Effects records tamoxifen as being implicated in the development of superficial corneal opacities, decreased visual acuity and retinopathy (Fraunfelder & Meyer, 1983). These effects seem to occur at higher doses of tamoxifen and are rarely seen until treatment has been ongoing for about 12 to 18 months. It has been proposed that excitotoxic effects in the retina are caused by an extracellular accumulation of glutamate. Glutamate uptake by pig retinal pigment epithelial cell cultures is reduced in the presence of tamoxifen in a dose-dependent fashion (Maenpaa et al., 1997). Cell culture studies have demonstrated that tamoxifen exposure causes a decrease in activity of lysosomal enzymes within the retinal pigment epithelium resulting in possible eye toxicity (Toimela et al., 1995).

The potential role of tamoxifen in the development of cataracts has been studied in isolated bovine lens fibre cells in culture. Opacification of the lens appears to be related to the effect of tamoxifen in blocking the chloride channels in the lens of the eye. These channels are essential for maintaining normal lens hydration and transmittance and for the process of volume regulation. Inhibition of the chloride channels results in inhibition of volume regulation, lens swelling and opacification (Zhang & Jacob, 1996). Tamoxifen caused lens opacity, associated with cataracts, at clinically relevant concentrations (Zhang et al., 1994) (Zhang et al., 1995).

3.6.2 Clinical evidence

Much of the clinical evidence on the ocular toxicity of tamoxifen is anecdotal and presented as case reports. An early report, published in 1978, described four patients who had received high dose tamoxifen for over a year, three of whom had a significant decrease in visual acuity as the result of a retinopathy accompanied by macular oedema. Unusual corneal changes were also evident (Kaiser-Kupfer & Lippman, 1978). Nineteen patients treated at normal dose levels for periods of 3 months to 4 years were also studied and no ocular changes were observed (Beck & Mills, 1979).

Other case reports document a variety of ocular effects including tamoxifen retinopathy (Gerner, 1989), bilateral optic neuritis (Pugesgaard & Von-Eyben, 1986), bilateral optic disc swelling and retinal haemorrhage after 3 weeks of treatment which were completely reversible on cessation of tamoxifen therapy (Ashford et al., 1988), bilateral intraretinal opacities, lesions at the level of the retinal pigment epithelium and cystoid macular oedema (McKeown et al., 1981). Prolonged low-dose therapy for 9 years has also been reported to cause bilateral retinopathy which was partially reversible after tamoxifen was stopped (Chang et al., 1992). A larger study of 135 visually asymptomatic tamoxifen treated patients identified only two patients with intraretinal refractile crystals, consistent with tamoxifen retinopathy. Corneal crystals, macular oedema and optic nerve changes were absent (Heier et al., 1994).

A cohort study involving 79 patients who had received tamoxifen in conventional dosage for varying treatment durations and 115 patients who had no tamoxifen exposure found no evidence of ocular toxicity (Longstaff et al., 1989). In 1992, a prospective study in 63 patients receiving treatment at a daily dose of 20 mg for a median duration of 25 months reported a variety of optical findings including decreased visual acuity, bilateral macular oedema, yellow-white dots in the paramacular and fovea areas and corneal opacities in 6.3% of patients. These effects,

apart from the corneal opacities, were reversible on cessation of treatment and suggest that there is ocular toxicity associated with long-term, low dose tamoxifen therapy (Pavlidis et al., 1992).

The only large study of ocular toxicity in patients receiving tamoxifen therapy within a RCT has been carried out within the NSABP B-14 study of adjuvant tamoxifen in the treatment of early breast cancer, in which patients were randomised to receive 10 mg tamoxifen twice a day, or placebo. The Tamoxifen Ophthalmic Evaluation Study (TOES) was performed in 303 women in this trial and within the TOES study there were women who had never received tamoxifen (n=85), women who had received tamoxifen for an average of 4.8 years and then been off treatment for an average of 2.7 years (n=140), and women who had been on tamoxifen continuously for an average of 7.8 years (n=78). Compared with untreated women, those who had received tamoxifen showed no difference in visual acuity or other tests of visual function, except colour screening. Intraretinal crystals (OR=3.58, p=0.178) and posterior subcapsular opacities (OR=4.03, p=0.034) were more frequent in the tamoxifen-treated group (Gorin et al., 1998).

4.1 Record Linkage System

In addition to the main Glossary, a description of the terms used in this chapter is provided in *Section 4.9*.

4.1.1 Background

The Scottish record linkage system provides a means of linking together computerised hospital discharge records (SMR1), cancer registration records (SMR6) and death records from the General Registry Office (GRO) belonging to the same patient (Kendrick & Clark, 1993). The idea was first conceived in 1967 when the decision was taken that all hospital discharge records, cancer registrations and death records would be held centrally in machine readable form and would contain patient identifying information such as names, dates of birth and areas of residence. Implementation of this system began in 1968.

A number of linkages were performed between the late 1960's and the mid 1980's, in which pairs of records were linked. In the late 1980's increased computing power and data storage facilities made possible the linking together of the set of records pertaining to a given individual and the linkage system was reconstituted. All SMR1, SMR6 and the GRO death record pertaining to a particular patient are grouped together and the resulting combined database, which currently consists of over 14 million records relating to over 4 million individuals, can be linked to external databases. The combined database is thus a source of person-based as well as episode-based data.

4.1.2 Quality checking of the combined database

Quality checking of the combined database by ISD has indicated that, on a pair-wise basis, both the false positive and the false negative rates are around 1%. However, the combined database is composed of groups of records, each group consisting of a primary record and all other records associated with that primary record. The larger

the group of records, the greater the possibility that it contains one or more records that do not belong to that individual. In particular, patient record sets containing a death record have been found to have a relatively high error rate. An ongoing clerical checking procedure aims to keep the overall proportion of patient record sets containing a mismatched record to around 1%.

4.1.3 Probability matching

The basis of the procedure, whereby the combined database is linked to an external database, is probability matching. A computer algorithm calculates a score or probability weight, for each pair of records (one from the combined database and one from the external database), which is proportional to an estimated probability that they belong to the same person. The overall score is the sum of the scores derived from the comparison of each item of identifying information. In principle, any item whose level of agreement or disagreement influences the probability that the two records do, or do not, belong to the same person can be used by the computer algorithm. However, items should, as far as possible, be statistically independent. For example, postcode of residence and hospital of referral are likely to be related and could not be used as independently contributing data items.

The data linking process can be conveniently divided into three steps:

- i) bringing the records together
- ii) calculating the probability weights
- iii) making the linkage decision.

The process has been described in considerable detail elsewhere (Kendrick & Clark, 1993, Kendrick, 1997).

The key to the calculation of probability weights is the odds ratio, this being the ratio of the frequency of a given outcome in pairs of records which relate to the same person and the frequency of a given outcome in pairs of records which do not belong to the same person. An odds ratio is calculated for every outcome of the comparison between the two records and combined, by multiplication, to give an overall odds ratio. Frequencies of outcomes are determined in the first place by a combination of previous experience and common sense. For example, the odds ratio for agreement of month of birth is around 12.

The probability weights derived do not represent absolute odds that the records concerned belong to the same person but rather are relative odds, which order the pairs in a particular linkage according to the likelihood that they belong to the same person. The relationship between relative odds and absolute odds depends upon several factors, including the way that the linkage is structured and this is one of the most important aspects of designing a linkage.

The absolute odds of a correct match can only be determined by clerical checking and the decision on the threshold probability weight to use in a linkage is usually determined by clerical inspection of a sample of pair comparisons across the weight range. Based on this, the decision of the appropriate threshold to use can either be completely automatic, or can use supplementary clerical checking within a 'grey zone'.

The discrepant spelling of surname is one of the most common reasons for mismatching identifiers in two records belonging to the same person. The procedure at ISD involves combining two different solutions to this problem. Soundex codes are generated by bringing together similar sounding consonants and ignoring vowels. The Soundex code is combined with the NYSIIS name compression algorithm, whereby other commonly miscoded elements of surnames are brought together. The resulting

Soundex/NYSIIS code is treated as for any other identifier. Account is taken of how frequently each code arises and the degree of letter-by-letter agreement between abbreviated surnames.

4.2 Confidentiality and Data Protection

Before the project was undertaken, a proposal was submitted to and approved by the Privacy Advisory Committee of the Information and Statistics Division. Submission to local ethics committees was not deemed necessary for this study. The use of named-patient data was required for the study and the data protection requirements were covered by the Data Protection Registration of the University of Edinburgh. It was necessary to use named-patient data in order to access relevant trial documentation and case-notes and due regard was paid to confidentiality at all times. The final database for statistical analysis did not include patient names. The named-patient data was archived, with password protection, and will be deleted in line with the request of the PAC when final review of the thesis is complete.

4.3 Linkage with the Scottish tamoxifen trial data

4.3.1 Procedure

The Scottish tamoxifen trial database was created and maintained on a relational database system (Knowledge Man v1.07, Micro Data Base Systems Inc.) at the Scottish Cancer Trials Office at the University of Edinburgh until November 1993. The trial notes and computerised database were then transferred to the Scottish Cancer Therapy Network in Edinburgh, for archiving.

Key variables on this database, which were to be matched against variables on the combined database during the linkage procedure, were exported into ASCII format onto a 3.5" diskette and sent to ISD where the linking process was undertaken.

The variables included for matching were:

Surname

First initial

Date of birth

Region of hospital referral at breast cancer diagnosis

(Aberdeen, Dundee, Edinburgh or Glasgow)

Date of randomisation

Date of death, if applicable.

The sex of all patients was known to be female and this was used in the matching algorithm. The information on region of referral was a stratification variable in the trial randomisation and was coded into the 7 digit trial number for each subject.

The linkage procedure carried out by ISD involved the comparison of trial records with records on the combined database and assigning a probability weight which, as already discussed, reflects the possibility that the records belong to the same individual. On the advice of the experts at ISD, a probability weight of 15 was chosen as that below which the chance of detecting a true match was minimal. If two linked records had a matching score of 15 or greater then they were retained on the linked dataset. In addition, the complete group of records associated with the primary linked record on the combined database was also added to the linked dataset. Linked records with a matching score of less than 15 were not added to the linked dataset.

4.3.2 Scope of the linkage output

The first output, containing the results of the linkage of Scottish tamoxifen trial records with the combined database, was received from ISD in November 1996. At that time, the combined database contained SMR1 records from 1981 to 30th June 1985, SMR6 records from 1980 to 31st December 1992, 90% of registrations for 1993 and approximately 60% of those from 1994. Death registration was complete

from 1980 to 31st December 1994. Recruitment to the trial started in 1978; therefore events occurring in 1978 and 1979 were not captured by the linkage process. Details of an update to the linkage are described in *Section 4.5*.

4.3.3 Processing of linked dataset

The linked dataset was received on 3.5" diskette as a compressed ASCII text file which was decompressed onto the hard drive of a pentium desktop computer. ASCII files are, by definition, unstructured and unformatted and, in order to facilitate utilisation of these data, it was necessary to import the file into a database or spreadsheet software package. A relational database was created in Microsoft Access and the data imported into this structure. A description of the fields created for the database is given in *Table 4.1* and sample output of the data after importing into the database is shown in *Figure 4.1*.

Table 4.1

FIELD NAME	DESCRIPTION
Misc1	Numeric identifier defining source of the data: 1 = SMR1 Record 6 = SMR6 record 9 = death record
Surname	Surname
Initial	First Initial
DOB	Date of birth in format yyyyymmdd
Misc2	Sex (2=Female) and Marital Status 1=single, 2=Married, 3=Widowed, 8=Other, 9=NK
Postcode	Postcode of address at trial entry
Dates	SMR1: dates of admission, discharge, outcome code SMR6: date of registration and ICD9 code of malignancy GRO: date and ICD9 code for cause of death
Proc_date	Date of procedure
Proc	Procedure
Cause	SMR1: ICD9 codes for reason for admission

Database field names and descriptions for importing the linked dataset

Figure 4.1

Misc1	Surname	Initial	DOB	Misc2	Postcode	Dates	Proc_date	Proc	Cause
34.38									
1 S226H2204225682	SMITH		W	200422	EH	850214 820930			
6	SMITH		WJ	19200422	EH123AB	1820929821009	820930	38219039	-1749
1 S226H2204225682	SMITH		W	19200422	EH123AB	820930 1742 8500			
1 S116H224417	SMITH		W	19200422	EH123AB	1821117821123	821118	3899	-1749
*1 S116H224417	SMYTH		W	19200422	EH123AB	1841112841116	000000		-1749
9	SMYTH		W	19200422	EH123AB	6850201850214	000000		-1749
18.98									
	SMITH		W	19200422	EH123AB	850214 17491990			
	SWAN		M	240204	DD	000000 830610			
*1 G504H234463X	SIM		MT	19240204	KA234DE	0831231831231	000000		-25009
1 G107H987588E	SIM		M	19240204	KA234DE	1850115850117	000000		-4439
1 G107H987588E	SIM		M	19240204	KA234DE	1850122850203	850123	0369	-4439
1 G107H987588E	SIM		MT	19240204	KA234DE	1850318850507	850319	8843	-7071
1 G107H987588E	SIM		M	19240204	KA234DE	1850625850701	850626	9169	-4439
1 A111H678178	SIM		M	19240204	KA234DE	5851105851109	000000		-1540
6	SIM		MM	19240204	KA234DE	851105 1536 8010			-4599
1 A111H678178	SIM		M	19240204	KA234DE	7851109851113	000000		-7991
6	SIM		MM	19240204	KA234DE	851113 1539 8000			-7991
9	SIM		M	19240204	KA234DE	851113 1539878658	6 2500		-1540
26.98									
	SWEENEY		F	330527	G	850327 831006			
*1 G104H057364X	SWEENEY		F MUIR	19330527	G 567JK	1831004831013	831006	3821	-1749
6	SWEENEY		F	19330527	G 567JK	831006 1749 8010			
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1840905840907	840906	0801	-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1841004841006	000000		-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1841026841027	000000		-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1841116841116	841116	9611	-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1841206841206	000000		-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1841228841228	841228	9611	-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JK	1850118850118	850118	9611	-1749
1 G107H712345V	SWEENEY		F	19330527	G 567JL	1850208850208	850208	9611	-1749

Sample output from linked data imported into MS Access database

Figure 4.1 shows clearly that the data are presented as groups of records, with the matching score, or overall probability weight, derived from the linking process, appearing in the column **Misc1** and in the row immediately preceding the first member of the group. This score is given as a numeric value to two decimal places and, as previously described, is a measure of the relative odds that the trial record and the record from the combined database with which it has been matched relate to the same individual. A single record in each group is marked with an asterisk (*). This is the primary record in the group which has been matched with the trial record. All other records in the group are linked to this primary record in the combined database and so they too are added to the linked dataset. However, it is important to remember that the matching score relates only to the relationship between the primary record and the trial record, and that no matching score is allocated to the other members of the group. It is known from the quality checking procedures at ISD that in up to 1% of groups in the combined database, one or more of the records belongs to a different individual. It is therefore essential that all records in a group be reviewed as part of the validation procedure, which is described in detail in *Section 4.4*.

The first row of each group, immediately below the matching score, contains the data relating to the patients in the tamoxifen trial and comprises, from left to right, surname, initial, date of birth, region of hospital referral (Aberdeen (A) Edinburgh (EH) Dundee (DD) Glasgow (G)), date of death (000000 if not applicable) and date of randomisation. Dates are all recorded in the format **yymmdd**, except for date of birth which records a 4 digit year. The remaining records in each group contain the data from the combined database which has been linked with that trial record. Although not illustrated in *Figure 4.1*, a single trial record may appear several times in the linked dataset, having been linked with different primary records with different matching scores. Only one of these linkages can be correct and this is assessed during the validation process (*Section 4.4*).

For SMR1 records the field **Misc1** includes a hospital case reference number. This is specific for each episode of hospital admission and one individual may have several different numbers. Therefore, this is not a particularly useful variable in the linkage validation process except that if it is identical for two records, then this is confirmation that the records belong to the same individual.

The information in the field **Dates**, varies depending on whether the source of the information is SMR1, SMR6 or GRO death records. For SMR1 records the field contains, from right to left, date of admission, date of discharge and a single digit code indicating outcome of admission (discharge home or to another hospital, death with or without postmortem, transfer to another speciality). For SMR6 records, **Dates** contains the date of registration, the ICD9 classification of tumour site and a procedure code. For GRO records, **Dates** contains date of death and ICD9 codes for cause of death.

For SMR1 records, the field **Proc_date** contains the date of any procedure (000000 if no procedure was carried out) and **Proc** contains the procedure code. These fields are both blank for SMR6 and death records.

The field **Cause** contains up to six reasons for admission to hospital for SMR1 records. The field is blank for SMR6 and death records.

4.3.4 Re-formatting of output structure

It is quite clear from *Figure 4.1* and the above description that the Access database created from the linked dataset has a variable record structure and is therefore not immediately suitable for manipulation and interrogation in the normal way. In order to produce a database that conformed to the conventional structure of columns (fields) and rows (records), it was necessary to reformat the structure. A conventional database structure was required so that records could be selected according to

particular specified criteria; e.g. those with a probability score of 20 or those with an ICD9 code of 4109 (myocardial infarction). None of the reformatting changes described below involved editing the data in any way; only structural changes to the database and the way in which the data are distributed within the database fields were involved. Visual checks against the original data file were made on an ongoing database to validate the changes. Master copies of the original data file and the original database, before the described structural edits were carried out, were retained for reference and archived under password protection (*Section 4.2*).

The main structural change made to the Access database was to create a field, **Score**, which was populated with the matching score for every record in a linked group. As has already been illustrated, the matching score in the original ISD output refers to the match of the primary record from the combined database with the trial record. If all records in the group refer to the same patient then that score is valid for every member of the group. However, as has already been described, 1% of groups or more contain a mismatched record. This field was therefore populated manually and due care was taken during the process to identify all possible instances of a mismatched record within a group. The occurrence of mismatches within a group is discussed more fully in *Section 4.4.1*. Validation of apparently discrepant members of a group was carried out using those checking procedures described in *Section 4.4.2*.

Table 4.1 also illustrates that SMR6 and GRO records differ from SMR1 records in the location of the ICD9 code which identifies the events. For this reason, the single digit identifying the source of the record (SMR1, SMR6, GRO) was split off into a separate field, **Type**. This enabled separate criteria searches to be instigated for SMR6 and GRO records.

The final structural change to the database was to create six discrete fields from the field **Cause**, in order to separately record each ICD9 code of reason for admission and thereby facilitate the search for relevant endpoints.

The final structure of the database was defined, in part by the original output received from ISD. It was not possible to attain a totally regular row structure as the first member of each group was necessarily the record from the Scottish trial data, which had a different field structure to that of the linked group from the combined database. An example of the final database for a single group of records is illustrated in *Figure 4.2*.

4.4 Linkage Validation

4.4.1 Mismatches within a group

Figure 4.2 also provides an illustration of the presence of a mismatched record within a group. It can be seen that all members of the group have the same surname, initial and date of birth but there are different hospital case reference numbers and postcodes. Hospital case reference numbers refer to particular episodes of hospitalisation and differences in this number are therefore not a valid criterion for discarding a match although, if they are the same, that is sufficient information to confirm a match. Postcode is dependent on address and may therefore change during the course of follow-up. In *Figure 4.2*, the date of hospital admission of *Record 14* is after the date of death in *Record 13*. These records clearly belong to different individuals. *Records 2-13* refer to the tamoxifen trial patient identified in *Record 1*, with agreement in the dates of breast cancer diagnosis/date of randomisation (*Record 3*) and date of death (*Record 13*). *Records 14-19* refer to a different individual, who is not a trial patient. The examples in *Figures 1 and 2* are based on real data but the details have been changed to protect the identity of the individuals concerned.

Figure 4.2

Score	Type	Misc1	Surname	Initial	DOB	Misc2	Postcode	Dates	Proc_date	Proc	Cause_2	Cause_3	Cause_4
25.29	1	*1 T101H4508200222	SYME	A	200711		DD	38072530529					
25.29	6	6	SYME	A	19200711	22	AD899JT	1810528810612	810529	3839	-1749		
25.29	1	1 T104H4508200222	SYME	A B	19200711	2	AD899JT	1851107851111	000000		-6811		
25.29	1	1 F709H009984	SYME	A	19200711	22	AD899HJ	1860627860627	860627	7041	-6271		
25.29	1	1 F709H009984	SYME	A	19200711	22	AD899HJ	1861109861119	861110	7129	-6181		
25.29	1	1 F709H009984	SYME	A	19200711	22	AD899HJ	1871106871106	871106	7041	-6271		
25.29	6	6	SYME	A C	19200711	2	AD899HJ	871106 1799 8951					
25.29	1	1 F709H069984	SYME	A	19200711	22	AD899HJ	1871129871211	871130	69696812	-1820		
25.29	1	1 T101H4508200222	SYME	A	19200711	22	AD899HJ	1880118880219	880122	9999	-1799		
25.29	1	1 F709H009984	SYME	A	19200711	22	AD899HJ	1880612880613	880613	7289	-6235		
25.29	1	1 T101H4508200222	SYME	A	19200711	22	AD899HJ	1880710880715	880712	7053	-1799		
25.29	9	9	SYME	A	19200711	2	AD899HJ	38072530529					
	1	1 F803H284536	SYME	E	19200711	29	NM134AA	38072530529			-5713		
	1	1 F701H015198	SYME	A	19200711	23	NM134AA	1901029901407			-5589		
	1	1 F805HMI24536E	SIME	A	19200711	29	AD580SF	1930704930708			-5990	-0389	-4969
	1	1 F704HV347006X	SIME	A	19200711	23	AD580SF	1941024941028			-5990	-0414	-0413
	1	1 F805HD456558M	SIME	A	19200711	29	AD580SF	7941103941106			-5579	-4149	-5712
	9	9	SIME	A	19200711	2	AD580SF	941106 5579440057	154149				

Record 1 – Date of Death (25/07/88)

Date of Randomisation (29/05/81)

Record 3 – Date of Diagnosis (29/05/81)

Record 13 – Date of Death (25/07/88)

Record 14 – Date of Admission (20/06/89)

No Matching Score assigned

Sample output from linked dataset after structural modification of the MS Access database

It is important to identify these cases of mismatches within a group in order to avoid false ascertainment of events. In the example shown, the individual, who we know is not a trial patient, is recorded as having suffered a myocardial infarction, which is one of the endpoints of the study. Failure to detect such mismatches could be a source of error in the study.

During all validation procedures, every effort was made to identify groups which contained one or more records incorrectly linked to the primary record matching the trial record. Changes in surname and postcode occur relatively frequently in the patient population in the study and where such changes were observed in a group of records, careful manual checking was carried out. Nevertheless, the only method of detection of mismatches within a group was by observation and incidental finding. Complete ascertainment cannot therefore be guaranteed. In *Figure 4.2* it can be seen that the records in the group that are mismatched with the primary record have not been assigned a matching score. This ensures that they will not be selected during the ascertainment of events which is described in *Section 4.6*.

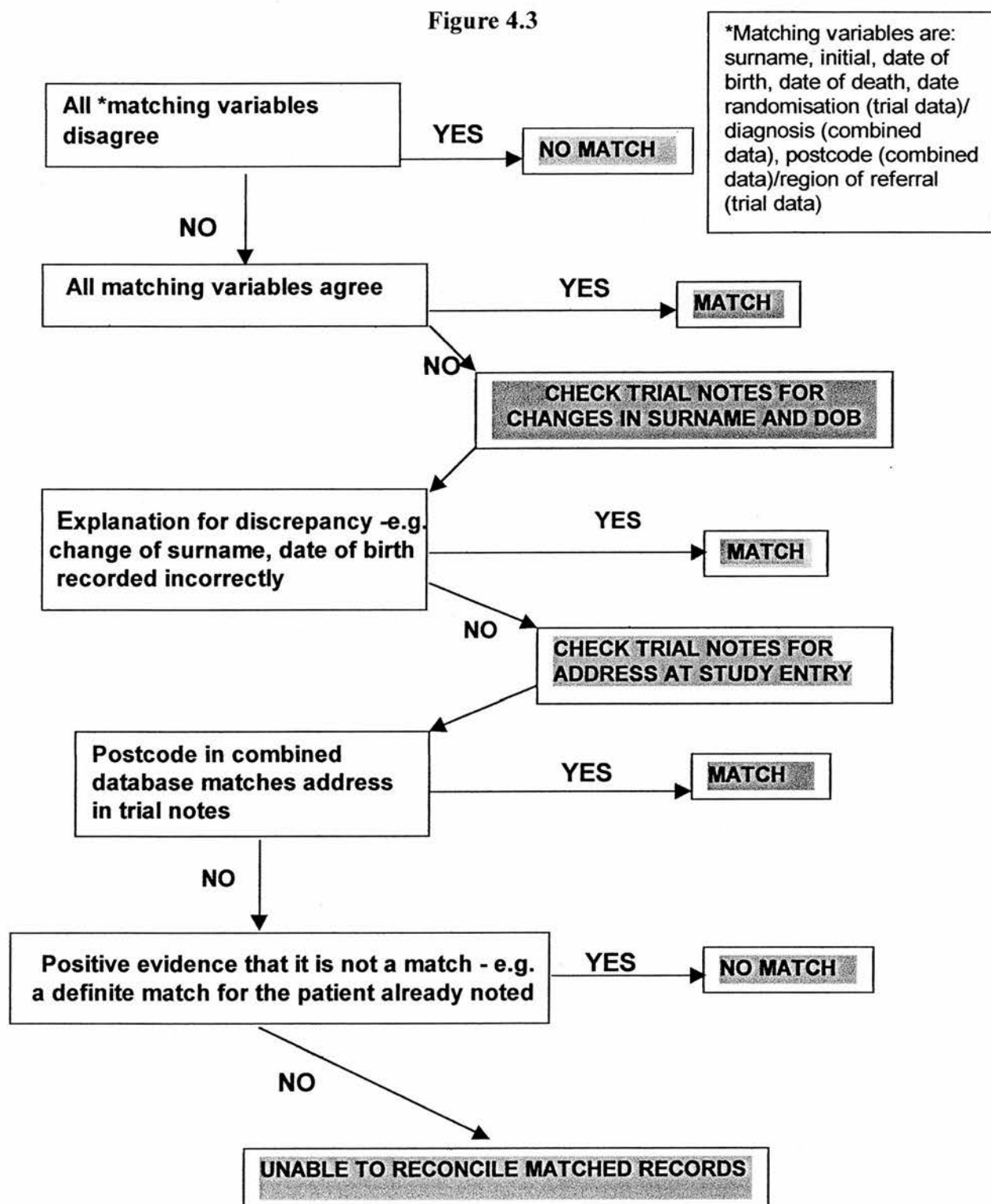
4.4.2 Clerical checking

All linked records with a matching score of 15 or more were included in the output received from ISD. Scores were given to 2 decimal places but were truncated at the whole number for the purposes of verification. It was not feasible, nor considered necessary, to check clerically all of these records and a decision had to be made which matches could be accepted or rejected without further checking.

From the previous experience of the author, working with output from similar data linkage procedures, it was known that matches with scores of 24 or more were robust and those of scores 16 or less were, for the most part, mismatches. An arbitrary decision was taken to check all matches with a score of 20 and to subsequently work progressively up from this score until the point was reached when the absolute odds of a true match were 95 / 5 – i.e. 95% of the matches were verified as true matches. All scores above this would be accepted as true matches without further checking. In the same way, scores of less than 20 would be progressively checked until the score which yielded 5% or less of true matches was reached. All matches with scores less than this value would be rejected as mismatches without further checking. In this way, the risk of including false positive matches or of excluding true matches was minimised while, at the same time, maintaining a manageable level of clerical checking.

All groups having a score of 20 were therefore printed out for manual checking as the first step in this process. Verification of the matches was done by following the procedure illustrated in the flow diagram (*Figure 4.3*). The process was complicated by the fact that it was not always possible to compare like with like. For example, the combined dataset contains a postcode, whereas the trial database only records the region of referral. Also, the trial database has the date of randomisation whereas the combined dataset has the date of diagnosis, which may or may not be the same as the date of randomisation.

Figure 4.3



If it was not immediately clear whether records were matched or mismatched, then further validation steps were required. This involved firstly checking the dates of breast cancer diagnosis and death which were recorded on the trial database against the SMR6 and GRO records, and secondly reviewing the trial notes, located in SCTN Central Office, to check date of birth, postal address and any surname changes. Date of birth is a variable that is commonly recorded inaccurately. The algorithm used to calculate the probability score is weighted heavily on date of birth as a matching variable and it therefore has considerable influence on the final matching score. An error in recording date of birth can therefore result in a true match having a falsely low probability score. These errors could often be detected by review of the documentation filed with the trial notes.

A further validation check was to compare the postcode provided on the linkage output against the address recorded on the trial notes at time of entry into the study. The postcode was not recorded on the trial database and so was not used in the linkage procedure, but it was requested on the linkage output for the purpose of providing additional verification. Its use was limited, however, in that the only address routinely recorded in the trial notes was that at the time of trial entry. Sometimes additional trial documentation highlighted a change of address that could be reconciled with the postcode included on the linkage output.

One of the most reliable criteria for discarding a match, and one that was used quite frequently, was if a trial record had an alternative matched record which could be positively verified. No cases were detected where two matches were provided and there was ambiguity about which of the matches was 'true' and which was 'false'. In every case, the 'true' match could be positively identified. Further, no cases were detected where two matches involving the same individual had a matching score which would automatically classify both as 'true' (>23).

One particular reason in this study for ‘true’ matches having a low probability score is related to the trial numbers allocated to those patients randomised into the Pilot B trial, which was one of the four constituent trials of the Scottish adjuvant tamoxifen trial. Pilot B was the earliest of the four trials and patients came from Dundee and Edinburgh. The trial numbers allocated to patients in this trial were not consistent with the algorithm used to determine the area of referral for patients in the other three trials; Trials B, C and D.

Although as standardised a procedure as possible was employed during the verification process, the efforts described above illustrate that, in addition to the formal procedures, keen observation was required and informed judgments had to be made. During the verification process, the author remained blinded to tamoxifen status.

4.5 Linkage updates

4.5.1 Scope of update

In April 1998, an update of the linkage between the tamoxifen trial database and the ISD combined dataset was obtained, both on disk and as hardcopy. This update included SMR1 data to 31st March 1996 and SMR6 and GRO death records to 31st December 1995 and this reflected the status of the combined database at this time.

4.5.2 Processing of linkage update

There were two possible strategies for processing the updated linkage. Firstly, a database could have been created and the linkage data imported into this and subsequently processed as already described. Comparison of the two databases would have identified the new data from the updated linkage. The second possibility was to process the updated linkage data from hardcopy listings. The decision was taken to proceed with the latter option, primarily because the procedures in Microsoft Access for comparing two databases and listing the discrepancies are cumbersome. Further,

the author already had considerable experience of working with hardcopy listings of linkage output and felt that a manual procedure could be implemented more quickly and effectively.

The hardcopy output was scrutinised and all events occurring in 1995 or later were highlighted. The matching score of each highlighted record was checked and matches were accepted or rejected as before on the basis of this score. Scores of >23 were accepted, scores of <18 were rejected and scores of $18 - 23$ were checked manually according to the criteria already described. Each accepted match was then reviewed individually and where any of the predefined endpoints under study were present, this record was added to the analysis database which is described in *Section 4.7*.

4.6 Ascertainment of 'Events'

After validation of the record matching was completed, the validated dataset was searched for records containing the ICD9 classifications shown in *Table 4.2*. These endpoints were predefined on the evidence of tamoxifen morbidity from the earlier studies and from the medical literature.

Table 4.2

Description	ICD9
Ischaemic Heart Disease (IHD): acute myocardial infarction (MI)	410
Other IHD (excluding acute MI) excluding 412 (old myocardial infarction)	411, 413, 414
Cerebrovascular Disease excluding 438 (late effects of cerebrovascular disease)	430-437
Thromboembolism: pulmonary embolism, phlebitis, thrombophlebitis	451, 415.1
Skeletal Effects: Fractures	800 – 829
Malignancy: primary neoplasms	140-195
malignant neoplasm of lymphatic and haematopoietic tissue	200-208
Ocular Effects: Disorders of the eye and adnexa	360-379
Dementias: Senile and presenile organic psychotic conditions	290
Benign Endometrial Disorders: endometriosis – uterus polyp of corpus uteri endometrial cystic hyperplasia	617.0, 621.0, 621.3

**Endpoints and their ICD9 classification, for the study of morbidity within the
Scottish adjuvant tamoxifen trial**

Note: where an ICD9 code is given to 3 digits, all further subclassifications are included e.g. 451 includes 4510-4519 inclusive

The study does not attempt to look at the incidence of contralateral breast cancers occurring within the Scottish adjuvant tamoxifen trial as the ICD9 coding does not allow differentiation of the original breast tumour from new disease in the contralateral breast.

Dementia was considered to be a valid and interesting outcome for study because, although there are little data relating tamoxifen use to dementia, there are data which indicate that postmenopausal oestrogen use can ameliorate the effects of Alzheimer's disease (Whitehead, 1996) or exert a protective effect on the risk of developing Alzheimer's disease (Kawas et al., 1997). Studies in the ovariectomised rat model have shown that oestrogen replacement is active in tests of learning and memory and activates basal forebrain cholinergic neurons and neurotrophin expression. In tissue culture, oestrogen is neuroprotective for human neuronal cultures (Simpkins et al., 1997). The level of interest in this topic is illustrated by the fact that cognitive function is one of the outcomes being studied in the Multiple Outcomes of Raloxifene (MORE) study of the drug raloxifene. This molecule has some oestrogenic and antioestrogenic actions and is currently marketed for the treatment of postmenopausal osteoporosis. Raloxifene and the MORE study are discussed further in *Chapter 12*.

For ischaemic heart disease, classification 412 (old myocardial infarction) was omitted, as was classification 438 (late effects of cerebrovascular disease) in the cerebrovascular disease category. These classifications do not allow determination of the date of the definitive causal event. In the identification of new primary tumours, only invasive malignant tumours are included – in situ cancers are excluded. The ICD9 classification of phlebitis and thrombophlebitis (451) is referred to throughout the text as deep vein thrombosis.

The database was searched by defining queries in MS Access for each of the endpoints of interest. Separate queries were required to search SMR1, SMR6 and

GRO death records, as already described, because of the structure of the database. The queries were generated in SQL (Structured Query Language) and some examples are included as *Appendix 3*. The use of cause of death and SMR6 data to determine events distinguishes this study from that previously published in which linkage was only with SMR1 and therefore, relevant events which were only notified as a cause of death could not be ascertained.

4.7 Analysis Database

A database (**Tambase**) was created in Microsoft Access containing a record for each of the 1312 tamoxifen trial patients. Key variables were imported directly into the analysis database from the original tamoxifen trial database, to avoid transcription errors, and updated as necessary. The fields and field descriptions of these key variables of the analysis database are shown in *Table 4.3*.

Table 4.3

Variable Name	Description
trlno	7-digit tamoxifen trial number
dob	date of birth
drand	date of randomisation
option	randomised option
dmr	date of systemic relapse
dd	date of death
tstart	date tamoxifen started
tstop	date tamoxifen stopped
d_fup	date of last information

Key variables on analysis database (Tambase) imported from original trial database

In addition to the variables in *Table 4.3* a field for each of the endpoints being studied was created in which to record the date of the event. If the event had occurred this field was populated otherwise it was left blank. The analysis database was updated manually with the relevant information, which was obtained by interrogation of the linked dataset to determine the incidence of the endpoints of interest.

Manual updating was necessary because of the different ways in which a patient was identified on the linked dataset and the analysis database. Patients were identified on the linked dataset by name and date of birth, while they were identified on the analysis database, which did not contain patient names, by their unique 7 digit trial number. Determination of a patient’s trial number from their name and date of birth was achieved by reference to a printed list which contained all three variables. If a

particular event had occurred, the date field for that event was populated with the date of first occurrence of the event, as noted by the date of admission to hospital, date of new primary cancer or date of death for SMR1, SMR6 and GRO death records respectively. Otherwise this field was left blank.

Two further checks were made as the analysis database was being updated. Firstly, the date of birth on this database was checked against the date of birth on the linked dataset, thus providing a further validation check of the linked dataset. Secondly, the date of the event was checked against the date of randomisation. If the event occurred prior to randomisation then it was not included on the database and the first occurrence of that event occurring after randomisation, if available, was entered onto the analysis database. If there was no subsequent recording of the event, then the date field in the analysis database was left unpopulated.

A number of calculated variables were also added to **Tambase**. This procedure is described in *Chapter 5, Section 5.2.3*. The complete structure of the resulting database is attached as *Appendix 4*.

4.8 Trial Follow-up

4.8.1 Procedures

Tamoxifen trial follow-up was performed routinely by staff at SCTO, on an annual basis, until transfer of the trial database to SCTN in 1993, at which time routine follow-up stopped. At the time of the previous publication (McDonald et al. 1995), for which linkage data to 31st December 1992 were used, there were 661 patients alive and free of systemic relapse for whom further information on tamoxifen start and stop dates, date of systemic relapse and date last seen or date of death were required. However, it was decided to update the follow-up information on all alive patients, including those for whom a date of metastases was recorded, in order to allow

analyses to be performed which did not use the onset of metastatic disease as a criterion for censoring.

New information on dates of patient follow-up, together with details of disease recurrence and tamoxifen status (duration, stopped, continuing) were kindly supplied by Dr. Helen Stewart on a Microsoft Excel spreadsheet. Data were available to 1996 or 1997 for most patients. The date of follow-up (**d_fup**), date of systemic relapse (**dmr**), date of death (**dd**) were extracted from the spreadsheet and added to the analysis database. New information on tamoxifen start and stop dates (**tstart**, **tstop**) dates were also added, using the assumptions described in *Section 4.8.3* to determine actual dates from the tamoxifen durations supplied by Dr Stewart.

During this updating process, a number of discrepancies were identified between the analysis database (**Tambase**) and the spreadsheet. These arose because of routine edits made to the original trial database since the data were downloaded to **Tambase**. The discrepancies were reviewed and resolved individually by the author and Dr Stewart, to ensure consistency was maintained between **Tambase** and the trial data on the spreadsheet. As far as possible, follow-up information was obtained at the anniversary of the patients' mastectomy. However, as time from mastectomy increased, so did the likelihood that the patient had been discharged from routine hospital follow-up into the care of her GP. In an attempt to elicit the most up to date information, follow-up details were obtained by Dr Stewart for times other than the anniversary dates. All available follow-up information was utilised, as it was considered that the ascertainment of these data was random and not related to the occurrence of a particular event.

The manual procedures, as described, for updating the analysis database were laborious but unavoidable, due to the availability of the data from a number of different sources. However, there is an advantage in that these procedures involve

checking and re-checking the data and are therefore likely to result in data of greater integrity and quality.

4.8.2 Definition of systemic relapse

For most of the analyses in the current study, patients were censored at systemic relapse. Censoring is discussed in *Chapter 5, Section 5.1.6*. The definition of systemic relapse used was in accordance with that defined in the protocol for the Scottish adjuvant tamoxifen trial and the current staging definitions in use when the trial was started (TNM Classification of Malignant Tumours, UICC, Third Edition, 1978) Metastasis to the ipsilateral supraclavicular fossa constituted local rather than distant spread. Whether disease in the contralateral breast was recorded as a new primary breast cancer or as metastatic relapse was according to arbitrary rules defined and implemented at the Scottish Cancer Trials Office. These rules stated that a cancer in the contralateral breast was a new primary cancer, rather than metastatic spread of the original tumour, if all of the following applied:

- i) this was the first recurrence of disease
- ii) the disease occurred more than one year after the initial cancer
- iii) there was no subsequent relapse of disease for at least one year

Disease occurring in the contralateral axillary or supraclavicular nodes, with no breast involvement, was always considered to be distant metastatic spread.

Forty-seven (47) new breast primaries in the contralateral breast are recorded on the trial database which conform to the definition above. One more occurred in a patient who had already experienced a new primary cancer of the cervix. A further six contralateral breast cancers were recorded, which did not conform absolutely to the

strict definition used by the Scottish Cancer Trials Office, but were nonetheless recorded as new primary breast cancers. Five of these were diagnosed before one year had elapsed after the initial breast cancer diagnosis. In one case, the patient died from other causes within one year of the diagnosis of the contralateral breast cancer. These 54 cases have therefore not been classified as metastatic disease and censoring has not been applied. Where contralateral relapse occurred only in the axilla or supraclavicular fossa, this has been deemed to be distant disease and censoring has been applied from the date of occurrence.

4.8.3 Assumptions regarding tamoxifen status

Knowledge of a patient's tamoxifen status at all time-points throughout the follow-up period is a key feature of the analysis and this requires recording of the tamoxifen start and stop dates on the analysis database. The dates themselves were not recorded on the spreadsheet from which trial follow-up data were obtained but tamoxifen duration, in months, was recorded together with information on whether therapy was continuing or the year in which it had stopped. In particular, a number of elderly patients who had been receiving tamoxifen treatment for a number of years had elected to stop during the period since the last follow-up. A number of assumptions were implemented in order to deduce the tamoxifen start and stop dates required for the analysis from the information available. All calculations were to the nearest month and the 15th day of the month was used in subsequent analyses.

- i) If tamoxifen start date and duration of tamoxifen are known, then the date tamoxifen stopped was calculated from:

(date started + duration).

If the date of last follow-up is before this calculated stop date, date of last follow-up was used.

- ii) If the tamoxifen duration is known and treatment was continuing at the last follow-up or the patient had died on therapy, the tamoxifen start date was calculated from:

(date of follow-up or date of death – duration)

This would be expected to coincide approximately with a recurrence date for patients in the control arm.

There were a number of cases where tamoxifen therapy of a known duration had stopped but neither start nor stop dates were recorded on the analysis database. These cases were reviewed individually and the necessary information obtained from trial records held by Dr Stewart.

4.9 Glossary

Refer also to main Glossary

SMR1	database of Scottish hospital discharge records
SMR6	database of Scottish cancer registration records
GRO death records	death certification as notified to the General Registry Office
combined database	combined database of SMR1, SMR6 and GRO records, held at ISD
linked dataset	dataset obtained by linking the combined database with the Scottish tamoxifen trial database
validated dataset	linked dataset after removal of those records, shown by verification procedures, not to be true matches
analysis database	database containing a record for each of the 1312
(‘Tambase’)	tamoxifen trial patients, with details of all the endpoints under study and their dates of occurrence, obtained from interrogation of the validated dataset

5.1 Overview of statistical methods

5.1.1 Survival analyses

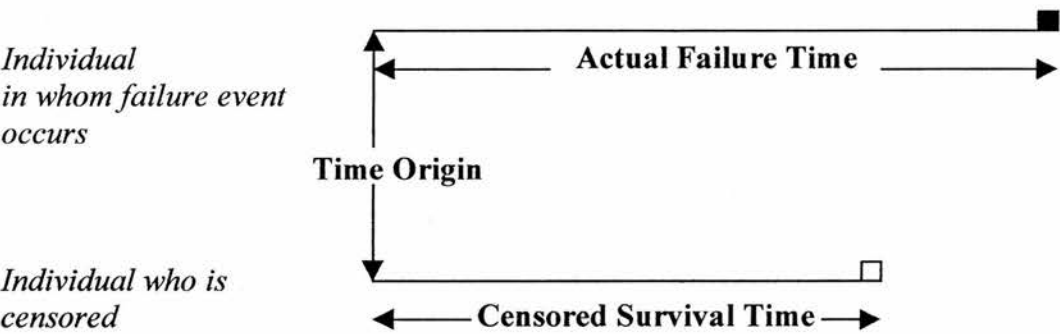
Survival analysis is a set of statistical techniques used in studies that investigate the occurrence of defined point events in time (or similar linear dimension), sometimes referred to as failure events. The data for analysis in this study take the general form of survival data and the failure time is the time from the time origin to the occurrence of the event under study. The time origin in this case is the date of randomisation into the study. Thus:

$$\text{failure time} = \text{time of failure event} - \text{time origin}$$

It is important that the key variable, failure time, is determined as reliably as possible. This requires a clear and unambiguous definition of the outcome of interest and the time origin of the study.

A key feature of the methods of survival analysis is the ability to deal with incomplete information. This most often occurs because observation has not continued until all individuals in the study have experienced the failure event; for example, because follow-up has stopped or because the individual has died from an unrelated cause. The method for allowing for incomplete information is known as censoring. This situation is represented in *Figure 5.1*

Figure 5.1



Schematic representation of censoring

Censoring is discussed in more detail in *Section 5.1.6*.

Survival data can be illustrated by a life table, which allows us to look at the time course of an event and calculate the probability of an event occurring at a particular time since the start of observation.

Table 5.1 below shows a very simple example of data for 45 women with breast cancer randomised into a clinical trial (Leathem & Brooks, 1987). The data are divided into two groups depending on whether the tumour was negatively or positively staining to a particular histochemical marker. The time origin is the date of surgery for breast cancer and the outcome of interest is death from breast cancer. Women who survived to the end of follow-up, or whose survival status was not known at the end of the study, were given a censored survival time to the date of last known follow-up. Similarly, women who died from a cause other than breast cancer were given a censored survival time. Censored survival times are denoted

by an asterisk (*) in *Table 5.1*. These data are used to illustrate some of the statistical methodology relevant to this study.

Table 5.1

GROUP 1 Negative staining Tumours		GROUP 2 Positive Staining Tumours
5	68	23
8	71	47
10	76*	69
13	105*	70*
18	107*	71*
24	109*	100*
26	113	101*
26	116*	148
31	118	181
35	143	198*
40	154*	
41	162*	
48	188*	
50	212*	
59	217*	
61	225*	

Survival times (months) for women in breast cancer study (Leathem & Brooks, 1987)

Table 5.2 illustrates the life table that has been derived from these data and the calculation of the Kaplan-Meier estimate of the survivor function ($S(t)$). This method of estimating the survivor function, also known as the product-limit estimate, utilises all the information that has been obtained from censored and non-censored individuals. To determine the Kaplan-Meier estimate of the survivor function from a sample of censored survival data, a series of time intervals is formed. Each of these intervals is constructed to be such that at least one failure time is contained in the interval and this failure time is taken to start at the start of the interval. It is assumed that deaths of individuals in the sample occur independently of one another. Note that no interval starts with a censored survival time. *Table 5.2* illustrates that censored individuals are no longer at risk of experiencing the event and the number at risk decreases by the sum of the number of deaths and the number of censored individuals.

The estimate of the survivor function, denoted by $S(t)$, is the probability of surviving to time t and is the product of the probabilities of surviving through all time periods preceding time t . $S(t)$ is a non-parametric estimator of the true survivor function; i.e. no assumption is made about the mathematical form of the 'true' curve $S(t)$. It can be shown mathematically that, assuming censoring occurs at random and survival times are measured exactly, $S(t)$ is a consistent estimator of the true survivor function and the estimate converges to the true value as the sample size increases.

The estimated survival function for the probability of surviving from 0 to t , where $t_j < t < t_{j+1}$

$$S(t) = \prod_{0 < t < t_{j+1}} (p) \times p_j$$

p_j = probability of surviving period j , conditional on having survived to time $j-1$, = $(1 - d/n)$

where d = number of deaths in period j , n = number at risk at start of period j

Table 5.2

Group 1:

Interval	Time	At Risk	Dead	Censored	Survivor Function
1	5	32	1	0	0.96875
2	8	31	1	0	0.9375
3	10	30	1	0	0.90625
4	13	29	1	0	0.875
5	18	28	1	0	0.84375
6	24	27	1	0	0.8125
7	26	26	2	0	0.75
8	31	24	1	0	0.71875
9	35	23	1	0	0.6875
10	40	22	1	0	0.65625
11	41	21	1	0	0.625
12	48	20	1	0	0.59375
13	50	19	1	0	0.5625
14	59	17	1	0	0.5
16	68	16	1	0	0.46875
17	71	15	1	4	0.4375
18	113	10	1	1	0.39375
19	118	8	1	0	0.34453
20	143	7	1	6	0.29531

Table 5.2 (continued)

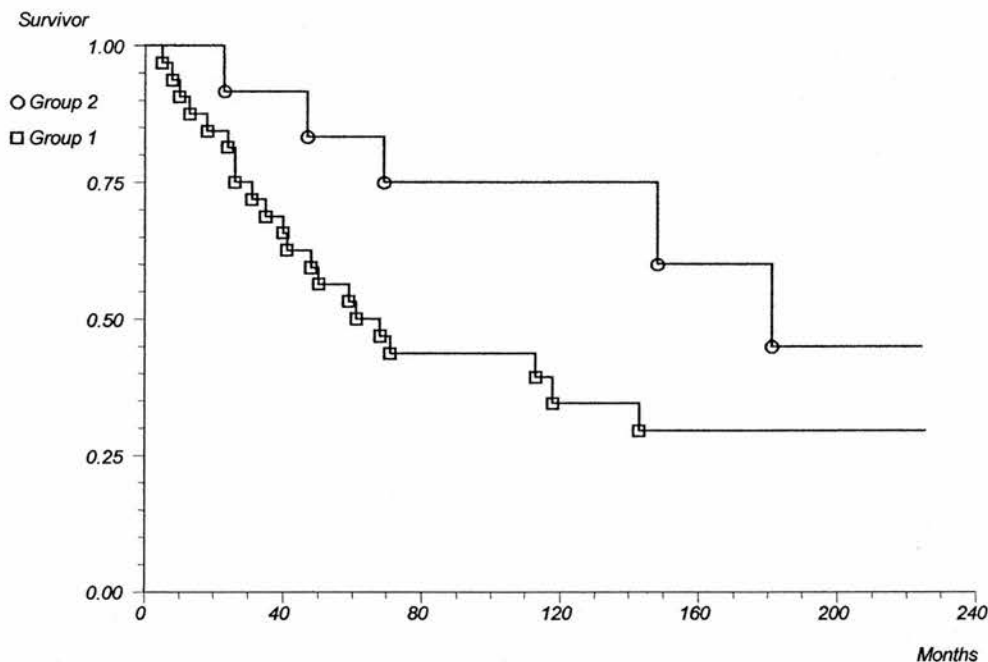
Group 2:

Interval	Time	At Risk	Dead	Censored	Survivor Function
1	23	12	1	0	0.91667
2	47	11	1	0	0.83333
3	69	10	1	4	0.75
4	148	5	1	0	0.6
5	181	4	1	3	0.45

Calculation of Kaplan-Meier estimate of survivor function for two groups of sample data in *Table 5.1*

These data can also be presented graphically in the form of a survival curve, by plotting the survivor function against the survival time. The survival curve for the data for all subjects in *Table 5.1* is presented in *Figure 5.2*

Figure 5.2



Survival curve for data in Table 5.1

5.1.2 Log rank test

The most common test for comparing the survival of two groups is the log rank test, also known as the Mantel-Haenszel test. This test compares the distribution of failures between two groups across the time-span of the study. It tests the null hypothesis that there is no difference in the survival experience of individuals in the two groups. The expected number of failures, assuming survival is the same in both groups, and the observed number of failures can be calculated for each group at each failure time. The test statistic is calculated by summing the deviation of

observed from expected frequencies, over all distinct failure times, and dividing by an estimate of the standard error of this total deviation. Thus, the test statistic summarises the extent to which the observed survival times in the two groups deviate from those expected under the null hypothesis of no group differences. The null hypothesis is rejected if the calculated value of the chi-square test statistic, with one degree of freedom, is very unlikely and we can conclude that there is a statistically significant difference in the survival experience between the two groups. An example of the calculation of the log rank test is given in *Table 5.3* for the sample data in *Table 5.1*.

Table 5.3

Failure Time (Months)	Deaths Gp 1= 0_1	n_1	Deaths Gp 2= 0_2	n_2	$d=0_1+0_2$	$n=n_1+n_2$	E_1	Variance V
5	0	13	1	32	1	45	0.2889	0.2054
8	0	13	1	31	1	44	0.2955	0.2082
10	0	13	1	30	1	43	0.3023	0.2109
13	0	13	1	29	1	42	0.3095	0.2137
18	0	13	1	28	1	41	0.3171	0.2165
23	1	13	0	27	1	40	0.3250	0.2194
24	0	12	1	27	1	39	0.3077	0.2130
26	0	12	2	26	2	38	0.6316	0.4205
31	0	12	1	24	1	36	0.3333	0.2222
35	0	12	1	23	1	35	0.3429	0.2253
40	0	12	1	22	1	34	0.3529	0.2284
41	0	12	1	21	1	33	0.3636	0.2314
47	1	12	0	20	1	32	0.3750	0.2344
48	0	11	1	20	1	31	0.3548	0.2289
50	0	11	1	19	1	30	0.3667	0.2322
59	0	11	1	18	1	29	0.3793	0.2354
61	0	11	1	17	1	28	0.3929	0.2385
68	0	11	1	16	1	27	0.4074	0.2414
69	1	11	0	15	1	26	0.4231	0.2441
71	0	9	1	15	1	24	0.3750	0.2344
113	0	6	1	10	1	16	0.3750	0.2344
118	0	6	1	8	1	14	0.4286	0.2449
143	0	6	1	7	1	13	0.4615	0.2485
148	1	6	0	6	1	12	0.5	0.2500
181	1	5	0	4	1	9	0.5556	0.2469
Total	5						9.5652	5.9289

Calculation of log rank statistic for data in Table 5.1

The variance, $V = d \times n_1 \times n_2(n-d) / (n^2 \times (n-1))$

The expected failure in Group 1 (E_1), at time t is calculated from:

$$\begin{aligned} & (\text{At risk in Group1/Total at risk}) \times \text{Total Failures} \\ & = (n_1/n) \times d \end{aligned}$$

$$\text{Extent of exposure to risk of death} = \sum E_1 = 9.565127$$

$$\begin{aligned} \text{Chi-square test statistic } \chi^2 &= (O_1 - E_1)^2 / V \text{ with 1 df} \\ &= (5 - 9.565) / 5.929 \\ &= 3.515 \\ P &= 0.061 \end{aligned}$$

For the data in *Table 5.1*, describing the survival experience of two groups of breast cancer patients, the P value is sufficiently small to cast doubt on the null hypothesis that there is no difference between the survivor functions for the two groups of women. However, the results are not statistically significant at the 5% level ($p > 0.05$)

This is illustrated in the survival curve in *Figure 5.2* in which the survival of both groups is plotted separately. The plot shows that, at any time t , the estimated probability of survival beyond t is greater for women with negatively staining tumours.

The log rank test for two groups can easily be extended to the comparison of three or more variables by calculating the observed and expected failures in each group at each of the distinct failure times. The test can therefore be used to look at the

effect of covariates with the limitation that the covariate must be categorised into groups, (e.g. age less than 45 years and age greater or equal to 45 years). Continuous variables cannot be incorporated into this model.

5.1.3 Hazard function

The hazard function at time t , $h(t)$, is defined as the probability that an individual will fail at time t , given that they have survived up to that point. This can be expressed as:

$$h(t) = f(t) / S(t) \text{ where } f(t) = \text{probability of failure at } t$$

$$S(t) = \text{probability of survival to time } t$$

The hazard is the rate of failure per unit time, and in order to estimate how 'risk' changes across time for an individual, we can look at the hazard at different points in time. This is illustrated in the survival curve in *Figure 5.2*. In the early part of the study, the survival curve is falling rapidly and the hazard is high. Towards the end of the follow-up period, the survival curve is virtually flat and the hazard is near zero.

5.1.4 Proportional hazards model

One model for the analysis of the hazard function of two groups of individuals is the proportional hazards model, proposed by Cox in 1972 (Cox, 1972). The model is referred to as a semi-parametric model, as no particular form of probability distribution is assumed for the survival times, and it provides a powerful and versatile tool in the comparison of survival between groups and the effect of prognostic variables on survival.

Implicit in this model is the assumption that the hazard at time t for an individual in group 1 ($h_1(t)$) is proportional to the hazard at the same time t for an individual in group 2 ($h_2(t)$); i.e. the ratio between them is independent of time.

Thus:

$$h_1(t) = \psi h_2(t) \quad \text{where } \psi \text{ is a constant}$$

The value of ψ is the ratio of the hazards at any time for an individual in one group relative to an individual in the other group and is known as the relative hazard or hazard ratio. If $\psi < 1$, then the hazard at time t for an individual in Group 1 is smaller than the hazard for an individual in Group 2. If individuals in Group 1 are on a new treatment and individuals in Group 2 are receiving standard treatment, then this is indicative that the new treatment is superior to the standard treatment.

Comparison of two groups

The proportional hazards model for the comparison of two treatments can be expressed as follows. Consider that X is an indicator variable, which takes the value 0 if an individual is on standard treatment and 1 if the individual is on the new treatment. If x_i is the value of X for the i th individual, then the hazard function ($h_i(t)$) for this individual can be written as:

$$h_i(t) = e^{\beta x_i} h_0(t)$$

where β is the log of the hazard ratio ($\beta = \log \psi$)

and $h_0(t)$ is the hazard function for an individual on the standard treatment

General form of the proportional hazards model

One of the objectives of modeling survival data is to determine how explanatory or indicator variables affect the form of the hazard function. These may be demographic variables, such as age and sex of the individual, physiological variables, such as various blood parameters or heart rate, or lifestyle variables such as diet and smoking history and they may be discrete (factors) or continuous

(variates). The proportional hazards model extends the methodology of the log rank test, by allowing incorporation into the model of multiple explanatory which may be factors, variates or a combination of the two types. The fundamental assumption of the model is that the effect of an explanatory variable on the hazard does not change with time (Tibshirani R, 1982). The effect of time-dependent covariates is discussed in *Section 5.1.5*.

As well as the inclusion of factors and variates in the model, it is possible to allow for the interaction between factors by incorporating an interaction term. This is necessary when the effect of one factor depends on the value of another factor.

The generalised form of the model for the values x_1, x_2, \dots, x_p , of explanatory variables X_1, X_2, \dots, X_p , can be expressed as

$$h_i(t) = \psi(x_i)h_0(t)$$

where $h_i(t)$ is the hazard function for the i th individual
and x is a vector representing the set of explanatory variables
such that $x=(x_1, x_2, \dots, x_p)'$

The hazard function $h_i(t)$ can also be expressed as

$$h_i(t) = \exp(\beta_1 x_{1i} + \beta_2 x_{2i} + \dots + \beta_p x_{pi}) h_0(t)$$

where $\beta_1, \beta_2, \dots, \beta_p$ are the coefficients of the explanatory variables

The function $h_0(t)$ represents the particular case where the values of all the explanatory variables that make up the vector x are zero and is known as the baseline hazard. No explicit form for $h_0(t)$ is required for this model. The β parameters can be estimated by the method of maximum likelihood (*Section 5.4*).

5.1.5 Time-dependent covariates

When explanatory variables are incorporated in a model for survival data, the values taken by these variables are those recorded at the time origin of the study. The basic assumption of the model is that the value of these explanatory variables is constant over time. An explanatory variable whose value changes over time and whose value is known at all times during the course of the study, is a time-dependent variable or updated covariate. Such variables can be incorporated in models used in the analysis of survival data such that the most recent value is used at each specific time in the modeling procedure (Altman 1994).

The general form of the Cox proportional hazards model, when some of the explanatory variables are time-dependent, can be written as:

$$h_i(t) = \exp \left\{ \sum_{j=1}^{j=p} \beta_j x_{ji}(t) \right\} h_0(t)$$

The values of the variables $X_{ji}(t)$ depend on the time, t and so the relative hazard, $h_i(t) / h_0(t)$ is also time dependent. In this model, the baseline hazard function, $h_0(t)$, is the hazard function for an individual for whom all the variables are zero at the time origin and remain at zero through time.

5.1.6 Censoring

As discussed in *Section 5.1.1*, censoring is a key feature of survival analysis and allows for the presence of incomplete information. Censoring may be an inherent feature of the design of a study, for example when the study ends at a pre-specified point, before failure has occurred in all individuals. This is Type I censoring and results in right-censored data, where in some individuals, the failure time is known only to be greater than a specified value. Inadvertent censoring, where the individual is lost to follow-up, withdraws from a study before failure or dies from

another unrelated cause also results in right-censored data. An alternative study design, less common in medical research, uses Type II censoring and the study ends when a pre-specified proportion of failures has occurred. This also results in right-censored data. There are also other, more complex types of censoring. In left-censored data, the failure time is known only to be before the most recent observation time and in interval-censored data the failure time is known to be at some time between the present and preceding observation times.

Censoring, like failure, is a point event and it is important that the timing of the occurrence of censoring is recorded as accurately as possible. An important assumption in survival analysis is that the censoring process is independent of individual survival times i.e. the likelihood of censoring is a random process and does not give any prognostic information about the subsequent survival time of that individual. This assumption is necessary so that the survival times of non-censored individuals can be used to estimate the survival times of censored individuals.

When the fact that censoring has occurred gives prognostic information about the individual and indicates that either a shorter or longer survival time is likely, this is described as **informative censoring**. Informative censoring introduces bias into the analysis and the standard methods of survival analysis are no longer valid. Care must be taken when defining censoring criteria that informative censoring is not being applied.

5.2 Statistical methods in the current study

5.2.1 Censoring

Right-censoring was applied in this study at whichever of the following occurred first:

- the date of last follow up information, after which no further information was available on disease or tamoxifen status

- 31st March 1996, after which no further linkage data were available.
- the date of diagnosis of distant cancer spread

Censoring was applied at the onset of metastatic disease in order to remove the confounding effect of the treatment of metastatic disease, particularly the use of chemotherapy, on the analyses of thromboembolic disease. The treatment of metastatic cancer with chemotherapy is a known, additional risk factor for thromboembolic events (Saphner et al., 1991). Censoring at metastatic relapse does not, however, guarantee the exclusion of patients receiving chemotherapy as, for example, some other cancers including new primary contralateral cancers may be treated by this modality. However, the number of subjects receiving chemotherapy and not being censored in the analyses is likely to be very small. Censoring at metastatic disease was applied in the earlier published studies and it was therefore deemed appropriate to use the same censoring criteria in this study.

In the original tamoxifen trial database, data on contralateral relapse in the breast, axilla and supraclavicular fossa was collected separately and arbitrary rules applied to define whether the disease was a new primary cancer or metastatic from the original tumour. In this study, 54 cases of cancer occurring in the contralateral breast have been considered to be new primary cancers and censoring has not been applied. Where contralateral disease occurrence was only in the axilla or supraclavicular nodes, these have been counted as distant disease relapse and censoring applied from the time of its occurrence.

It can be difficult, in the absence of histological evidence, to definitely classify a cancer, occurring at a site distant from the original breast tumour, as a new primary tumour rather than metastatic spread of the original breast tumour. This is particularly so when metastatic spread of the original tumour is already known to have occurred and therefore censoring at metastatic relapse is relevant when

considering the incidence of new primary cancers. Censoring at metastatic relapse with regard to the analyses of fractures and ocular toxicity is discussed further in *Section 5.7*.

5.2.2 Ascertainment of events

In this study, a number of analyses have been performed for different failure events; hospitalisation for myocardial infarction, other IHD, thromboembolism, CVA, fractures, ocular toxicity and non malignant endometrial pathologies. Registration of a new primary malignancy on SMR6 is also a failure event. These failure events were ascertained by interrogation of the validated linked dataset as already described in *Chapter 4*. Apart from fatal events, the outcomes being considered in this study can potentially be experienced by an individual on multiple occasions. The time origin for the study is the date of randomisation into the Scottish adjuvant tamoxifen trial and therefore any event occurring prior to randomisation was not included in the analyses. However, the patient was still considered to be at risk of experiencing a similar event until censored according to some other criteria, such as metastatic disease or the end of follow-up. For all analyses, only the first occurrence after randomisation, of an event was counted and subsequent episodes were disregarded.

As a result of the problem in differentiating new primary tumours from metastatic spread, it has been recognised that a diagnosis of cancer is sometimes inappropriately registered as a new primary cancer on SMR6. In an effort to ensure that only true new primary malignancies were included and cases of metastatic spread were excluded, a new primary cancer registration on SMR6 was only counted as an event provided there was no subsequent metastatic relapse diagnosed in the following 30 days.

5.2.3 Data manipulation and creation of variables

The data relating to study endpoints for 1312 patients in the tamoxifen trial were available in Microsoft Access on the analysis database (**Tambase**), which has already been described (*Chapter 4, Section 4.7, Appendix 4*). All data manipulations were performed within this database, before export for statistical analysis. In addition to variables already described a number of calculated variables were added to **Tambase** for the purposes of the analyses. These are described in *Table 5.4*. The values of these variables were calculated by running update queries in Microsoft Access and examples of these are attached as *Appendix 5*. Date of censoring was calculated as a separate variable and this was used, together with information on the date of occurrence of failure events, to determine whether the event had occurred (event=1) or the individual was censored (event=0).

Table 5.4

Variable	Variable Name	Description
Date of censoring	d_censor	Earliest of the following: Date of metastases if present Date of death if dead with no metastasis (cause other than study endpoint) Last follow-up date if alive and disease free (no further information on disease or tamoxifen status available) 31 st March 1996 if earlier than any of the above (no further linkage data available)
Time to tamoxifen start	start	Number of days from randomisation to tamoxifen start
Time to tamoxifen stop	stop	Number of days from randomisation to tamoxifen stop
Age	age	Age in years at randomisation calculated from (date of randomisation - date of birth)
Event	event	Takes the value 1 if the event has occurred and 0 if the individual is censored
Survival time	survtime	Number of days from randomisation to occurrence of the event (event=1) or to censoring (event=0)

Additional variables created in the analysis database

5.3 Preparation of data for analysis

The variables **age**, **option**, **start**, **stop**, **event** and **survtime** were exported from the analysis database (**Tambase**) into an ASCII text file and from there imported into Arcus Quickstat Biomedical (Research Solutions) for the log rank analysis and production of survival curves and cumulative log hazard curves. The same ASCII text file was imported into the software package EGRET (Serc) for the Cox proportional hazard analyses. Two additional variables, **t0** and **t1** were created during definition of the analysis file in EGRET. The variable **t0** was set to 0 and **t1** was set to 1. These are time-dependent variables that allow changes in tamoxifen status over time to be included in the statistical model. When an individual changes tamoxifen status, from no treatment to treatment started to treatment stopped, there is switching between the two time dependent variables **t0** and **t1**. For example, at entry to the study, before tamoxifen treatment is started, the variable **t0**, which always takes the value 0, is invoked. At the start of tamoxifen therapy, the variable **t1** which always takes the value 1, is invoked. In the analysis of current tamoxifen therapy versus not current, the variable switches again to **t0** when tamoxifen therapy is stopped.

This is illustrated in hypothetical example in *Table 5.5*. The number of days elapsing between randomisation and starting and stopping tamoxifen are shown, together with the switching of the time-dependent covariate at these time points to allow the tamoxifen status of each individual at all times during the study to be defined. Individuals who did not receive tamoxifen therapy have days to tam start and stop completed with '9999' and the variable **t0** is invoked throughout. Failure time is the number of days from randomisation to the event, or censoring. The value of the time dependent variable at failure time depends on which analysis ('Ever'/'Never' or 'Current'/'Not current') is being undertaken.

Table 5.5

Subject	Initial tam status	Tam start (days)	Tam status at start	Tam stop (days)	Failure time	Tam status at failure	
						Ever / Never	Current/ Not Current
001	t0	58	t1	2178	2178	t1	t1
002	t0	9999	t0	9999	5861	t0	t0
003	t0	76	t1	1095	3965	t1	t0
004	t0	9999	t0	9999	3643	t0	t0
005	t0	9999	t0	9999	5856	t0	t0
006	t0	547	t1	951	545	t1	t1
007	t0	0	t1	4280	5854	t1	t0
008	t0	0	t1	1282	1279	t1	t1
009	t0	27	t1	1812	5843	t0	t0
010	t0	9999	t0	9999	2724	t0	t0

**Description of tamoxifen status and illustration of switching between time
dependent variables t0 and t1**

t0 describes a non user of tamoxifen (never used or stopped use)

t1 describes a current user of tamoxifen

5.4 Implementation of the Cox model

The Cox proportional hazards model was implemented in EGRET for each of the endpoints being studied. Fitting the proportional hazards model to an observed set of survival data involves estimation the unknown coefficients of the explanatory variables (β). The values of β can then be used to construct an estimate of the

baseline hazard function. The failure-time variable indicates the point at which the individual corresponding to that observation either reached an endpoint or was censored. The failure-time variable in these analyses is **survtime**. The failure/censoring indicator variable, **event**, indicates whether the failure-time variable is a study endpoint or whether it is a censored observation.

The method of maximum likelihood was used to estimate the β coefficients. This method estimates the value of the coefficient that is most likely on the basis of the observed data. The likelihood function of the sample data is the joint probability of the observed data as a function of the observed failure times and the unknown β parameters. The maximum likelihood is the value of the likelihood function when parameters are replaced by their maximum likelihood estimates. The basic assumption used in the construction of a likelihood function for the proportional hazards model is that intervals between successive failure times convey no information about the effect of the explanatory variables on the hazard of death.

For comparing alternative models fitted to an observed set of survival data, a statistic which measures the extent to which the data are fitted by a particular model is required. For a given set of data the larger the value of the maximum likelihood the better is the agreement between the model and the observed data. In comparing alternative models it is more convenient to use minus twice the logarithm of the maximised likelihood ($-2 \log L$). As the maximum likelihood, L , is the product of probabilities, this statistic will always be < 1 . Therefore, $-2 \log L$ will always be positive and for a given data set, the smaller the value of $-2 \log L$, the better the model. Two models can be compared on the basis of the difference between the $(-2 \log L)$ statistics by calculation of the Likelihood Ratio Statistic (LRS):

Likelihood Ratio Statistic = $-2\log \{L(1)/L(2)\}$

where L(1) and L(2) are the maximised likelihood functions for Models 1 and 2 respectively

This statistic has an asymptotic chi-squared distribution with the number of degrees of freedom being equal to the difference between the number of parameters being fitted in the two models.

5.5 Statistical Analyses

For each endpoint, three separate analyses were performed:

Analysis 1 As Randomised.

Those randomised to tamoxifen compared with those randomised to observation.

Analysis 2 ‘Ever’ versus ‘Never’ use of tamoxifen.

Those ever exposed to tamoxifen (either current or previous) compared with those who have never had tamoxifen treatment

Analysis 3 ‘Current’ versus ‘Not current’ use of tamoxifen.

Those who are currently receiving tamoxifen at the time of the event compared with those who had either stopped or had never received treatment

Analysis 1 involves the comparison of two randomised groups and it can be reasonably assumed that the process of randomisation will result in an equal distribution of prognostic variables, such as tumour size, between the groups. Further, the study was stratified at randomisation for two important prognostic variables, axillary node status and menstrual status. The variable **option**, which defines the treatment assigned to the individual by randomisation, is entered into the analysis as a regression term.

Analyses 2 and 3 are observational and so no assumptions can be made about the distribution of prognostic variables. In this respect, they are less reliable than Analysis 1. For Analyses 2 and 3, the tamoxifen status is defined and included in the analyses for all women at all times up to censoring or failure time, by invoking the time-dependent covariates t_0 and t_1 .

In this study, the time origin for all analyses is the date of randomisation into the Scottish adjuvant tamoxifen trial.

5.6 Expression of Results

The results of the analyses are expressed as hazard ratios and 95% confidence intervals. The significance of the most recent model extension is measured by the likelihood ratio statistic. Where only one term is included in the regression, the likelihood ratio statistic measures the significance of adding this term to the null model. The null model is the special case of the proportional hazards model in which there are no explanatory variables and the hazard function is equal to the unknown baseline hazard, which is the same for all individuals.

5.7 Supplementary Analyses

Effect of Age

The analyses were all repeated with age, as a continuous variable, being entered into the model as the first regression term. The model was then extended by adding the variable **option** (Analysis 1) or the time-dependent variable describing tamoxifen status (Analyses 2 and 3). This allows the effect of treatment group and tamoxifen status to be determined, having taken account of the effect of age on the various endpoints.

Ocular Outcomes

There is no rationale for censoring at metastatic relapse when considering the incidence of ocular events. Therefore, an additional set of analyses of ocular toxicity was carried out, in which there was no censoring at metastatic relapse.

Fractures

It has been suggested that metastatic breast cancer, to sites other than the skeleton, is associated with generalised disturbances in skeletal metabolism with an increased risk of vertebral fracture. A 20-fold increase in the incidence of vertebral fracture has been seen in patients with soft tissue metastases (Kanis et al., 1999). Censoring at metastatic relapse for the outcome of fracture can therefore be considered as informative censoring, where the fact of metastatic disease is itself associated with the risk of experiencing the failure event. A further set of analyses was therefore carried out in which censoring at metastatic relapse was not applied but the presence or absence of metastatic disease was included as a time-dependent covariate. This covariate had the value 0 in the absence of metastatic disease and the value 1 after the onset of metastatic relapse.

Effects on the endometrium and dementias

The causal effect of tamoxifen on endometrial cancer has assumed great significance in the ongoing debate on the use of tamoxifen in the chemoprevention of breast cancer. However, endometrial cancer is rare and the number of cases in this study is small, so these results have not been subjected to statistical analysis but are described in *Chapter 6, Section 6.4*. Similarly there are small numbers of benign endometrial events and dementias and these results are also treated descriptively.

6.1 Validation of matched records in linked dataset

6.1.1 Matching scores

The linkage between the Scottish adjuvant tamoxifen trial and the combined database of SMR1, SMR6 and GRO death records produced an electronic file containing about 18,500 individual records. All the primary matched records output to the file had a matching score of at least 15, this being the relative odds that the record belonged to the same patient as the corresponding record in the trial database. As it was not possible to predetermine the probability weight required to give favourable absolute odds that the records belonged to the same patient, all scores of 17, 18, 19, 20, 21, 22 and 23 were checked manually as described in *Chapter 4, Section 4.4*.

The results of this validation exercise are shown in *Table 6.1*. The decision had been taken that when a matching score had less than 5% of true matches, all matches with a score less than this would be discarded. Conversely, when a matching score had 95% or more of true matches, representing an absolute odds of a true match of 95/5, all matches with a score greater than this would be accepted. Only 3.2% of matches with a score of 17 were found to be correct by manual checking and therefore, all matches with scores of 16 or less were discarded. 97.4% of records with a matching score of 23 were found to be correct and so all matches with a score of 24 or more were accepted. In all cases, it was possible to definitely categorise a match as ‘true’ or ‘false’.

There was one incidental finding of a trial record being matched with a record from the combined database with a matching score of 24 which was positively confirmed as an incorrect match, by virtue of having dates of hospital admission after the date of death of the trial patient. This group of records was therefore removed from the validated dataset.

Table 6.1

Matching Score	No. of groups on linked dataset	No. correct after validation	% correct matches
17	125	4	3.2
18	51	4	7.8
19	36	9	25
20	19	2	10.5
21	9	3	33.3
22	4	2	50
23	39	38	97.4

Note: sample size for a scores of 21 and 22 are very small (9 and 4 groups respectively).

Number and percentage of manually validated records with matching scores of 17-23 inclusive

After verification of the matches as described, the final validated, linked dataset contained a total of 9,968 records. A total of 1,237 of the 1,312 trial patients had been matched with a record from the combined database and its associated matched groups of records. As previously explained, a group of records on the validated dataset consists of the tamoxifen trial record, the record from the combined database most likely to match with it and all records in the combined database associated with this primary matched record.

6.2 Tamoxifen trial follow-up

Follow-up for patients in the Scottish adjuvant tamoxifen trial was obtained as already described (*Chapter 4, Section 4.8*) for all 1312 tamoxifen trial patients. Follow-up to at least 1996 was available for all but 23 of the alive patients and to 1993 for all but 7 alive patients. At the time of the current analysis, 751 patients were recorded as having died, 24 were alive with metastatic disease and 537 were alive and free of metastatic disease. The median follow-up for 561 patients recorded as alive is 178 months with a range of 1-232 months.

6.3 Ascertainment of ‘Events’

6.3.1 Numbers of events

The events identified in the validated, linked dataset are shown in *Table 6.2*, by ICD9 classification and randomised option (tamoxifen or observation). This table shows both the total number of events ascertained and the number of events included in the statistical analyses (in parenthesis). Events which occurred prior to the date of randomisation are not included in *Table 6.2*.

Each ICD9 code is counted only once for each patient. For example, if a patient suffered two instances of myocardial infarction (410.9), then only the first occurrence is included in *Table 6.2*. This is also true for ischaemic heart disease and cerebrovascular disease where only the first occurrence of an event in these classifications is counted. In the case of thromboembolism, the two endpoints of deep vein thrombosis and pulmonary embolism have been combined and therefore the number of events in *Table 6.2* represents the number of patients experiencing at least one episode of either of these events.

The situation with regard to fractures and new primary malignancies is different and patients are included in *Table 6.2* more than once if they had multiple occurrences of the events at different sites. For example, if a patient suffered two fractures at different sites having different ICD9 codes, then both are tabulated. Similarly for

new primary malignancies, *Table 6.2* therefore shows numbers of tumours rather than numbers of patients. The ocular endpoints were also ascertained individually and so multiple occurrences of ocular events are included.

The numbers in parenthesis in *Table 6.2* refers to the number of events that are included in the statistical analyses for each of the outcomes being studied. For the statistical analyses, only the first occurrence of an event in each Event Description is recorded for each patient and thus the occurrence of multiple fractures, new primary malignancies and ocular events occurring in one patient are not accounted for. For the primary analyses, censoring was applied at the time of metastatic relapse and events occurring after censoring are not included in the statistical analyses.

Table 6.2

Event Description	ICD9 Code	Tam	Control
Ischaemic Heart Disease (IHD) acute myocardial infarction (MI)	410	26 (20)	41 (29)
Other IHD (excluding acute MI) excluding 412 (old myocardial infarction)	411, 413, 414	39 (35)	38 (32)
Thromboembolism: pulmonary embolism, phlebitis, thrombophlebitis	451, 415.1	23 (17)	21 (11)
Cerebrovascular Disease excluding 438 (late effects of cerebrovascular disease)	430-437	44 (40)	31 (22)
Skeletal Effects: fractures	800 - 829	49 (31)	51 (33)
Malignancy: primary neoplasms leukaemia and lymphoma	140-195 200-208	87 (59)	70 (45)
Ocular Effects: Disorders of the eye and adnexa	360-379	62 (49)	34 (26)
Dementias: Senile and presenile organic psychotic conditions	290	9 (7)	9 (8)
Benign Endometrial Disorders: endometriosis - uterus polyp of corpus uteri endometrial cystic hyperplasia	617.0, 621.0, 621.3	9 (9)	2 (2)

Total number of events identified from the validated, linked dataset, by ICD9 classification and randomised option.

Number of events included in statistical analyses, after censoring for metastatic disease, is in parenthesis ()

6.3.2 Distribution of events

Table 6.2 shows very similar numbers of events in the two randomised arms of the trial for the endpoints of ischaemic heart disease, thromboembolism, fractures and dementia. For the endpoint of myocardial infarction, there are more events in the observation arm compared with the adjuvant tamoxifen arm (41 and 26 respectively). The numbers of events for the endpoints of cerebrovascular disease, ocular events and new primary malignancies are greater in those randomised to receive tamoxifen than in those randomised to observation. The total number of benign endometrial events and dementias is small (11 and 18 respectively).

6.3.3 Fractures

Table 6.3 shows a breakdown of the fractures by site. As previously described, a patient may appear in this table more than once, if they experience fractures at more than one site. Only the first fracture at any site will be included in the statistical analysis. The overall number of fractures in *Table 6.4* is similar in both arms of the trial, as is the distribution at each of the individual sites. The greatest number of fractures occurring at a single site is fractures of the femur, with 47 recorded cases.

6.3.4 New primary malignancies

Table 6.4 shows a breakdown of the incidence of new primary malignancies, excluding breast cancer, by site. Overall there are 87 new primary malignancies in patients randomised to receive tamoxifen compared with 70 in the observation arm. There is the suggestion of an increased incidence of new primary malignancies of the digestive organs in patients randomised to receive adjuvant tamoxifen (26) compared with those randomised to observation (14). This is also true of endometrial cancers (8 in the tamoxifen arm compared with 3 in the observation arm) and, but the total number of these events is very small. The group of genitourinary cancers, excluding endometrium, is also very small, but there is a suggestion of fewer occurring in those patients randomised to tamoxifen (6 compared with 11 in the observation arm).

Table 6.3

Site of fracture	ICD9 Code	Tam	Control
Face bones	802	0	1
Vertebral column	805	4	0
Ribs, sternum, larynx, trachea	807	2	2
Pelvis	808	6	5
Humerus	812	1	3
Radius and ulna	813	4	3
Carpal bone	814	0	1
Femur	820,821	22	25
Tibia, fibula	823	2	4
Ankle	824	6	5
Tarsal, metatarsal bones	825	2	2
TOTAL		49	51

Fractures identified in the validated, linked dataset, by ICD9 classification of site and randomised option

Table 6.4

Site of primary malignancy	ICD9	Tam	Control
Lip and oral cavity	140 - 149	1	1
Digestive organs	150 - 159	26	14
Respiratory and intrathoracic	160 - 165	15	16
Bone, connective tissue and skin	170 - 173	18	21
Genitourinary (ex. Endometrium - 182)	179 - 189	6	11
Endometrium	182	8	3
Other and unspecified sites	190 -195	8	2
Lymphatic and haematopoietic tissue	200-208	5	2
TOTAL		87	70

**New primary malignancies identified in the validated, linked dataset, by ICD9
classification of site and randomised option**

6.3.5 Ocular toxicity

Table 6.5 shows the breakdown of ocular events by cause. As with fractures and new primary malignancies, patients are included more than once in this table if they have experienced different ocular endpoints with different ICD9 codes. In the statistical analysis only the first ocular event experienced by a patient is included. Generally, the number of ocular events identified is low and there is a similar distribution of events between the two randomised arms of the study. There was however a high incidence of ocular cataracts (ICD9 Classification 366) with a total of 56 events being identified. The majority of these occurred in patients randomised to receive tamoxifen (38 events compared with 18 events in the observation arm). The recorded incidence of low vision and visual disturbances (ICD9 Classification 368, 369) is higher in the tamoxifen group than the observation group (6 compared 3) and there are more cases of glaucoma in the observation group (7 compared with 4). However the number of these events is small.

Table 6.5

Description	ICD9 Code	Tam	Control
Retinal detachments and defects Other retinal defects	361, 362	3	0
Disorders of the iris and ciliary body	364	1	0
Glaucoma	365	4	7
Cataract	366	38	18
Visual disturbances	368	2	1
Blindness and low vision	369	4	2
Keratitis	370	2	1
Disorders of conjunctiva	372	2	0
Inflammation of eyelids Other disorders of eyelids	373, 374	2	2
Disorders of lacrimal system	375	2	1
Strabismus and other disorders of binocular eye movements	378	1	1
Other disorders of eye	379	1	1
TOTAL		62	34

Ocular events identified in the validated, linked dataset, by ICD9 classification
and randomised option

6.4 Statistical Analyses

6.4.1 Log rank analysis by randomised option

Table 6.6 shows the hazard ratios, 95% confidence intervals and p values for the log rank analysis by randomised option (adjuvant tamoxifen versus tamoxifen at first relapse) for the failure events of myocardial infarction, other ischaemic heart disease, thromboembolism, cerebrovascular accident, new primary cancers, fractures and ocular toxicity.

Unlike *Tables 6.2* to *6.5*, which derive their data from the validated, linked dataset, the data for statistical analysis are derived from the analysis database (**Tambase**), in which only the first occurrence of an event corresponding to an Event Description in *Table 6.2* (e.g. IHD, fracture, new primary malignancy) is recorded. The date of the event is the date of admission to hospital for the first occurrence of the event, or the date of death if the event is noted only as a cause of death.

Results are statistically significant when $p < 0.05$ and the 95% confidence interval of the hazard ratio does not include unity. The p value is that obtained from the likelihood ratio statistic and it should be noted that it is theoretically possible, using this method, to have a p value of < 0.05 but a confidence interval which crosses unity. The use of the log rank test to compare the failure times of the adjuvant and observation groups is equivalent to the use of the Cox proportional hazards model with the randomised option as a single covariate. Survival curves are given for these analyses in *Figures 6.1 – 6.7*.

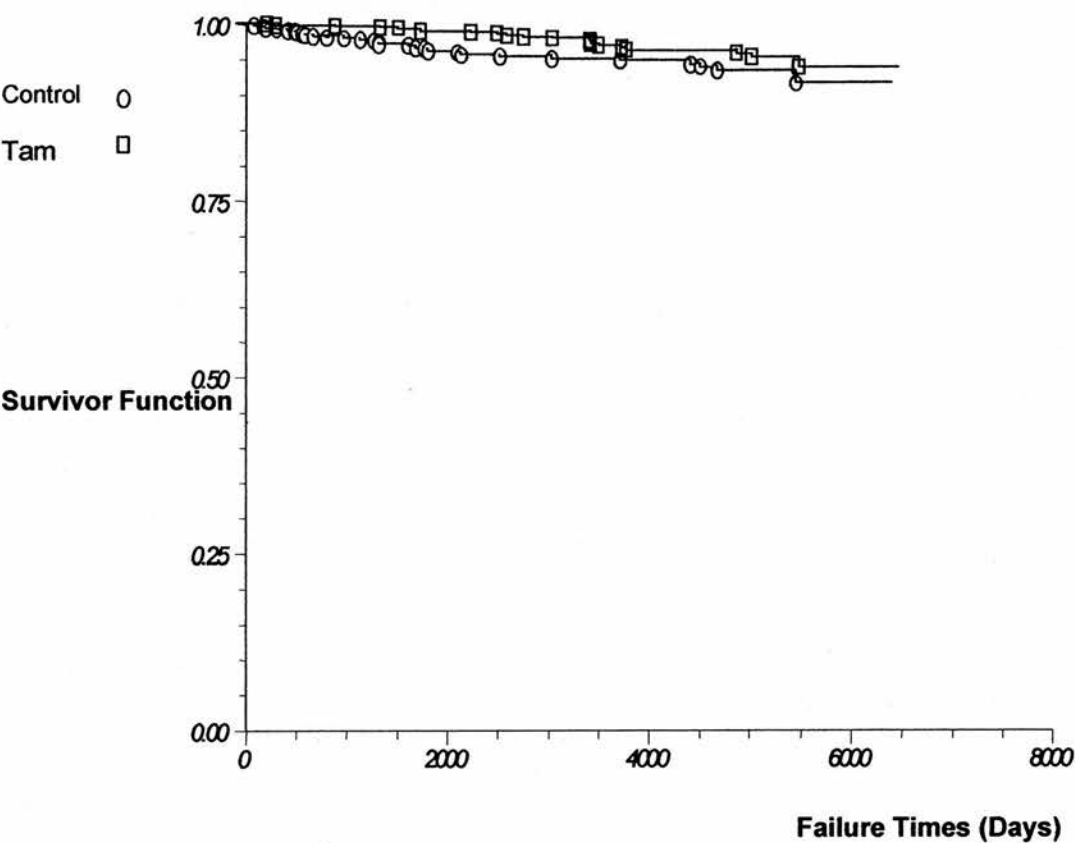
Table 6.6

Outcome	No. of events		HR*	95% CI	p
	Control	Tam			
MI	29	20	0.580	0.331 – 1.017	0.058
IHD	32	35	0.900	0.556 – 1.456	0.666
Thromb	11	17	1.319	0.627 – 2.773	0.472
CVA	22	40	1.533	0.930 – 2.527	0.105
Cancer	45	59	1.112	0.756 – 1.635	0.592
Fracture	33	31	0.775	0.474 – 1.268	0.306
Ocular	26	49	1.605	1.019 – 2.527	0.049

Log rank analysis comparing the survival experience of the tamoxifen group with the observation group, as randomised

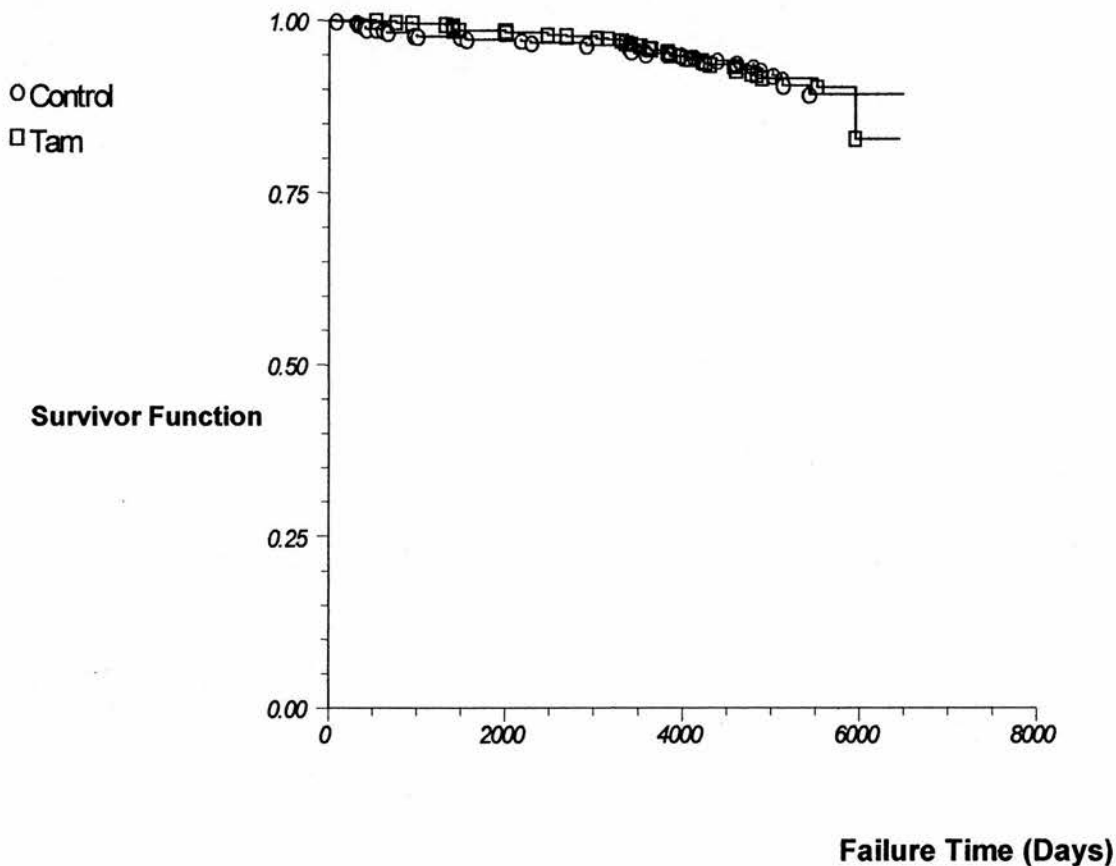
*Hazard Ratio (HR) is for tamoxifen arm compared with control

Figure 6.1



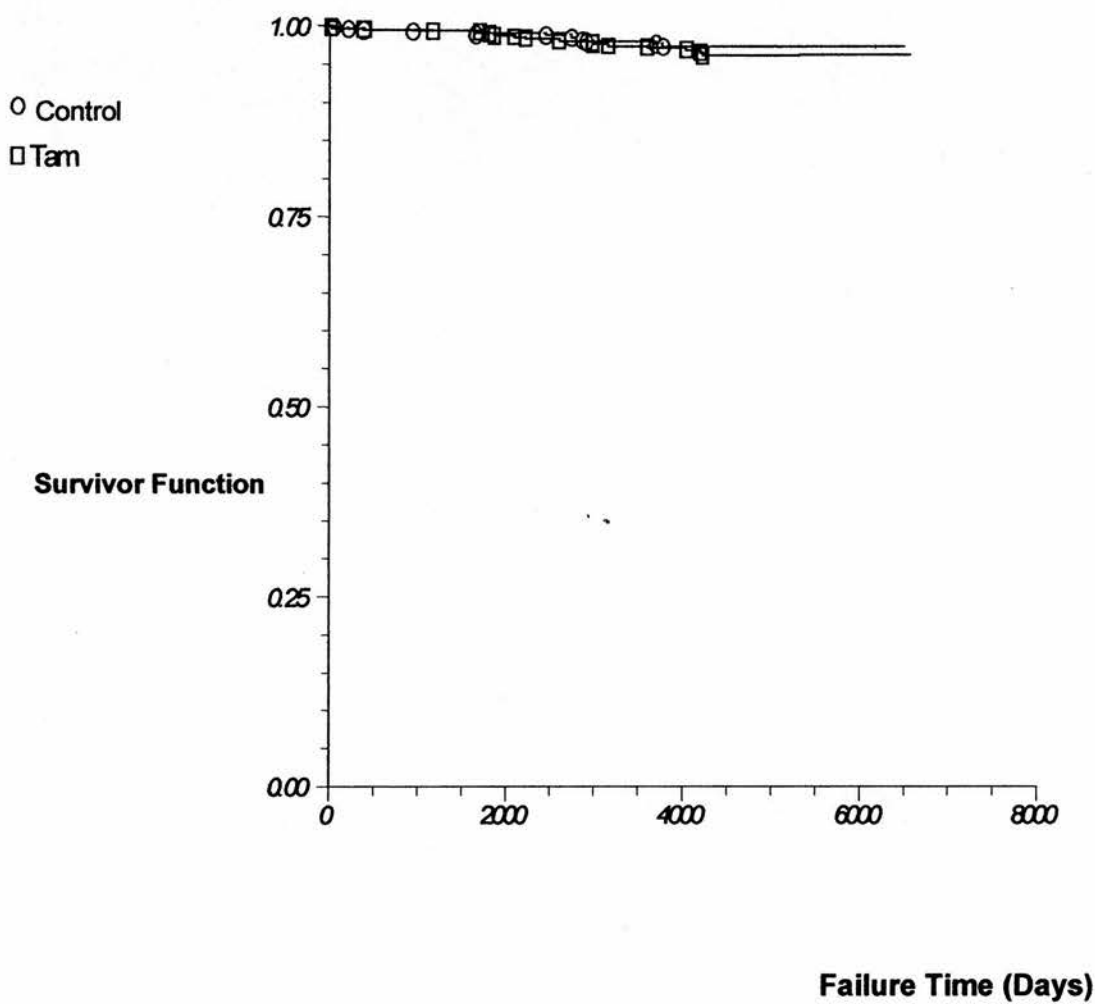
Survival curves showing rate of MI in the two randomised groups

Figure 6.2



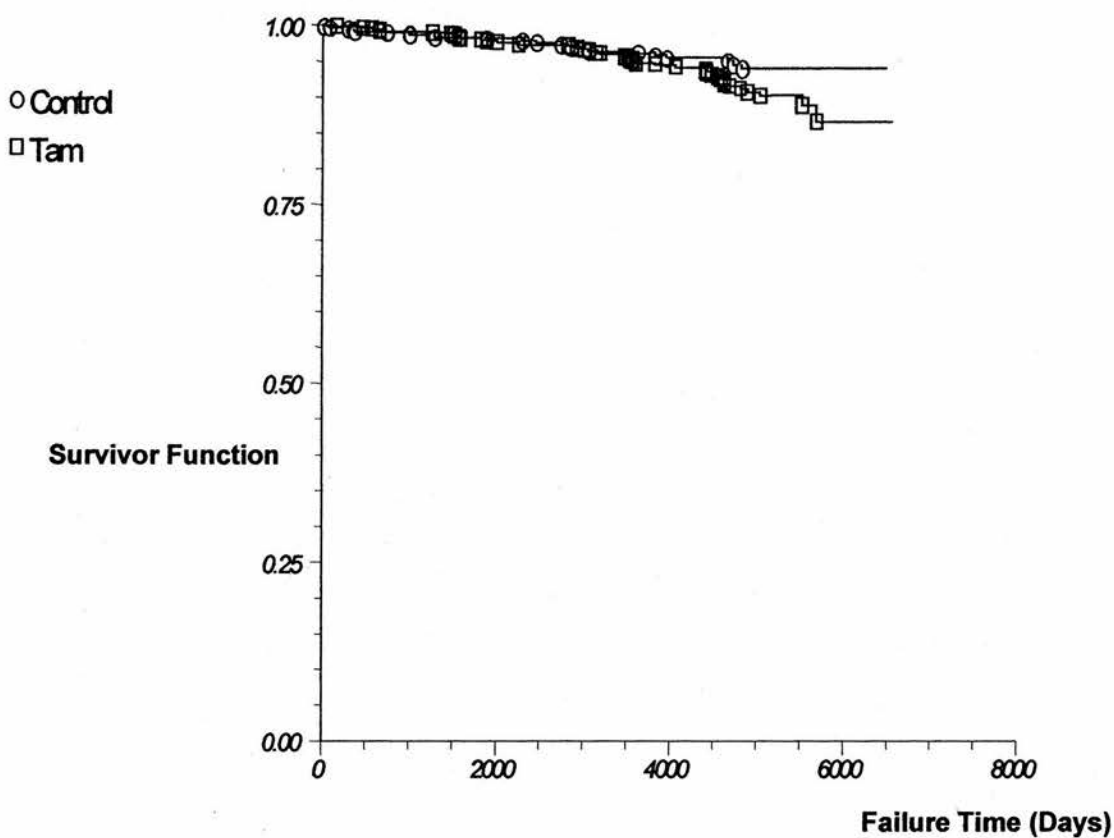
Survival curves showing rate of IHD (other than MI) in the two randomised groups

Figure 6.3



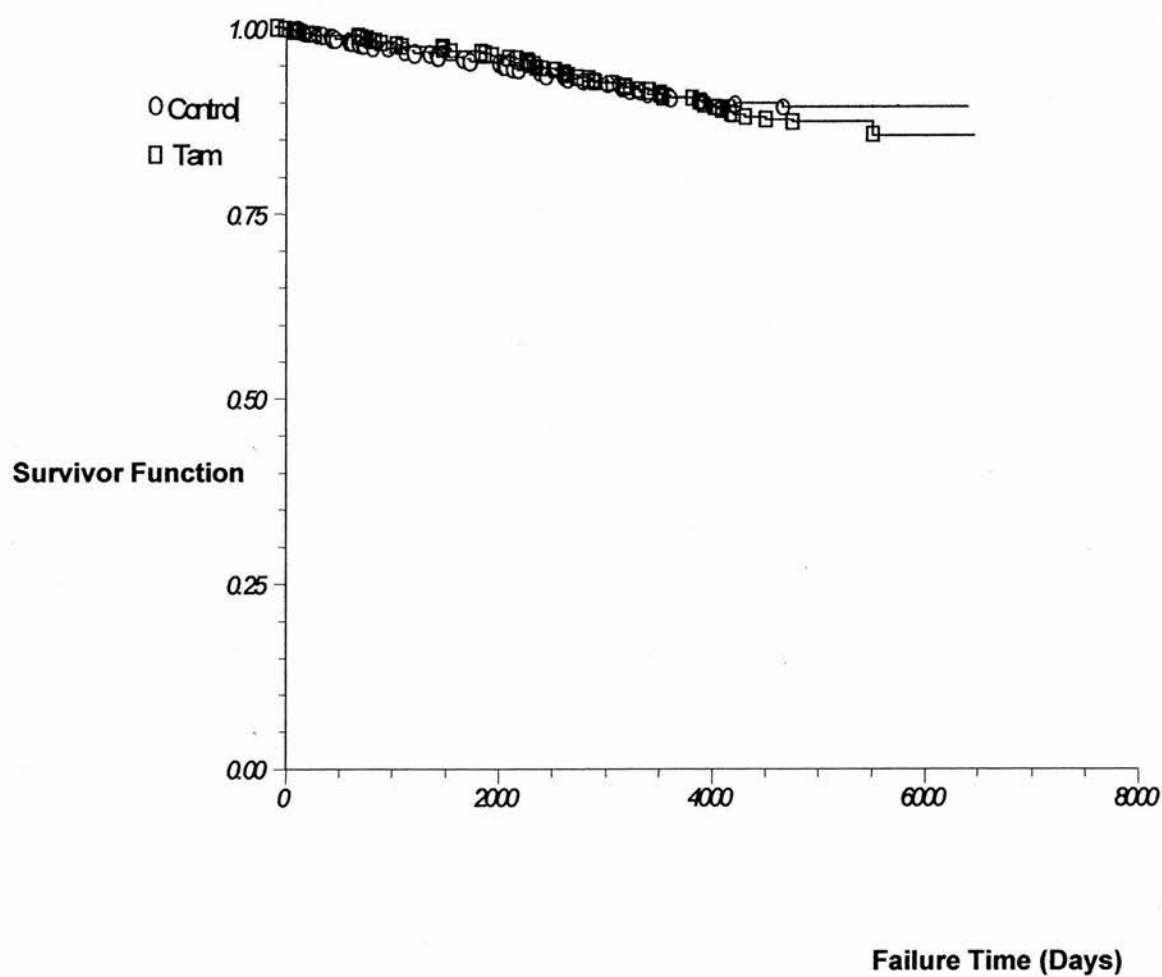
Survival curves showing rate of PE/DVT in the two randomised groups

Figure 6.4



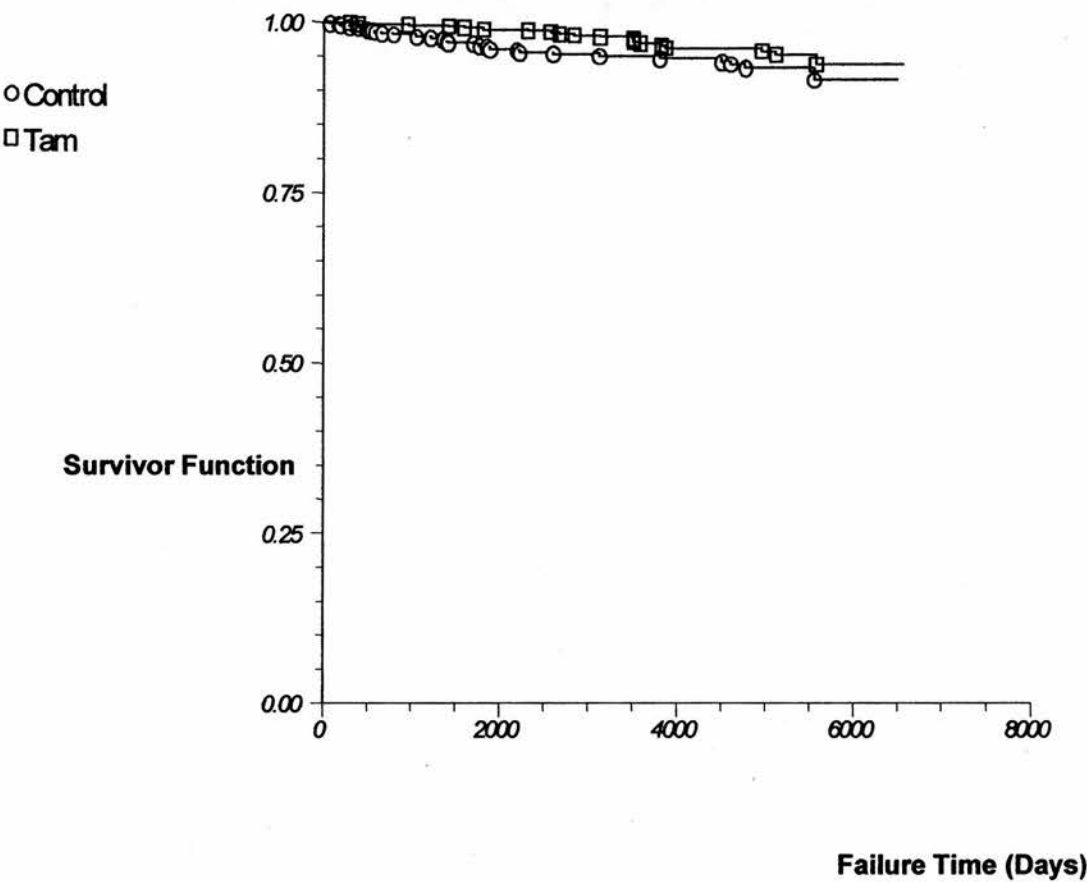
Survival curves showing rate of CVA in the two randomised groups

Figure 6.5



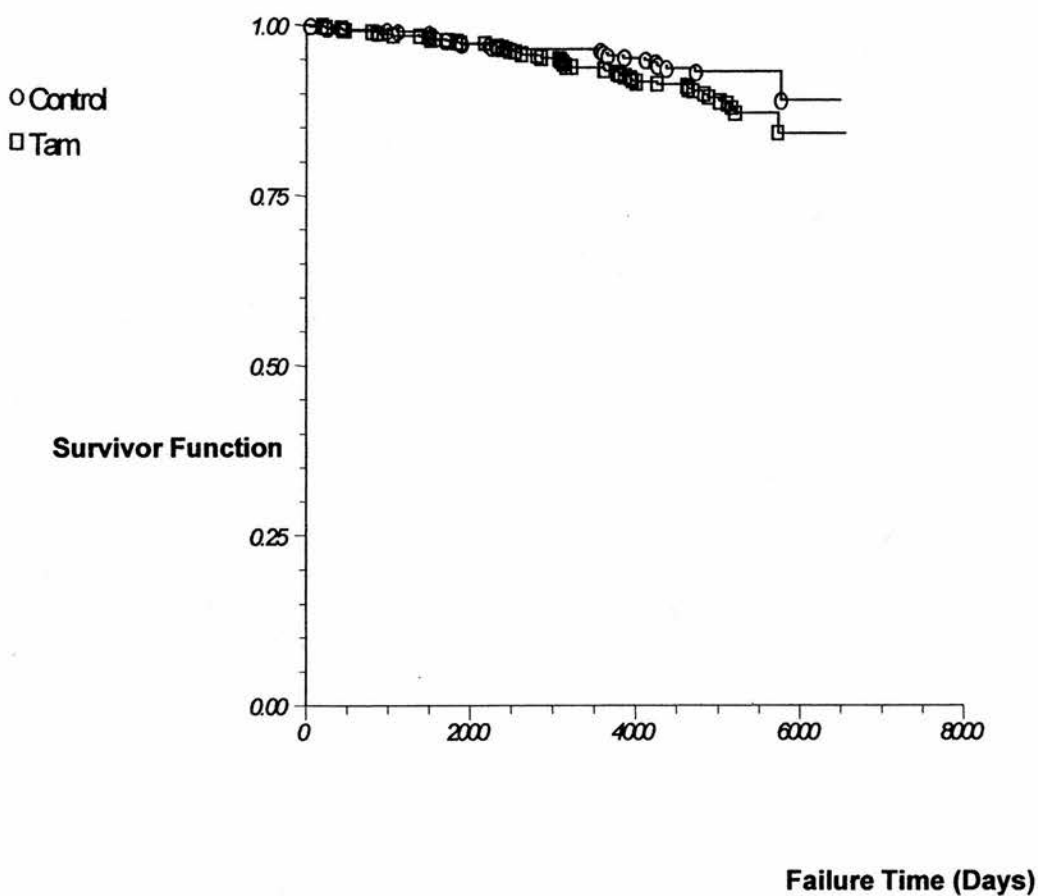
Survival curves showing rate of new primary cancers in the two randomised groups

Figure 6.6



Survival curves showing rate of fracture in the two randomised groups

Figure 6.7



Survival curves showing rate of ocular outcomes in the two randomised groups

The results show no significant differences between those randomised to receive tamoxifen and those randomised to observation for any of the failure events studied, except a borderline significantly increased incidence of hospital admission for ocular events in those patients randomised to tamoxifen ($p=0.049$).

6.4.2 Cox proportional hazards analyses

Table 6.7 presents the results, using the Cox proportional hazards method, of the analyses by randomised option, ‘Ever’ vs ‘Never’ and ‘Current’ vs ‘Not current’ use of tamoxifen. For the analyses by randomised option the results show some differences compared with the log rank method for the outcomes of MI, THROMB, CVA and OCULAR. These results are discussed further in *Chapter 7, Section 7.1*.

The results of the analyses of the ‘Ever’ vs ‘Never’ and ‘Current’ vs ‘Not current’ use of tamoxifen show a significantly reduced risk of admission to hospital for myocardial infarction in those patients currently receiving tamoxifen compared to those who have either stopped or never received tamoxifen ($p=0.034$). There is no significant difference in risk between the ‘Ever’ and ‘Never’ use groups. There is no significant difference in any of the analyses in the risk of admission for IHD other than myocardial infarction.

The risk of admission for a thromboembolic event (pulmonary embolism or deep vein thrombosis) is significantly increased in those currently receiving tamoxifen compared to those not receiving current treatment ($p=0.038$). There is no difference in thromboembolic events between ‘Never’ and ‘Ever’ use groups.

In the analyses of CVA, there is a significant difference between ‘Ever’ and ‘Never’ use groups ($p=0.007$). However, these results were not maintained in the analysis of current tamoxifen therapy compared with previous therapy or no exposure. There were no significant differences evident in any of the analyses relating to the endpoints of fracture or new primary malignancy.

In the analyses of ocular toxicity there was a significantly increased incidence of hospital admission for patients randomised to tamoxifen compared to those in the observation arm ($p=0.049$) in both the analysis by the log-rank method and that by the proportional hazards method ($p=0.024$). A similar, but more pronounced effect was seen in the comparison of those currently receiving tamoxifen compared with those who had stopped, or had never received, treatment ($p=0.006$).

The number of endpoints for the events of endometrial cancer, benign endometrial events and dementia are small and have therefore not been subjected to statistical analysis. These results are described in *Section 6.4*.

In all of the analyses one record is rejected as being a non-positive survival time. This was identified as a patient who was inappropriately entered into the tamoxifen trial. Metastases were diagnosed on the day of randomisation and the survival time is therefore 0.

Table 6.7

EVENT	N	RANDOMISED		EVER v NEVER		CURRENT v NOT CURRENT	
		HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
MI	49	1.051 (1.1033-1.083)	0.002	0.621 (0.354 – 1.091)	0.097	0.511 (0.267 – 0.976)	0.034
IHD	67	0.897 (0.558 – 1.443)	0.583	0.831 (0.513 – 1.347)	0.455	0.754 (0.444 – 1.279)	0.288
THROMB	28	1.056 (1.019-1.093)	0.046	1.848 (0.810 – 4.218)	0.130	2.229 (1.040 – 4.777)	0.038
CVA	62	1.079 (1.038 – 1.122)	0.013	2.169 (1.196 – 3.934)	0.007	0.848 (0.455 – 1.481)	0.557
FRACTURE	64	0.773 (0.474 – 1.263)	0.282	0.858 (0.523 – 1.406)	0.545	0.807 (0.473 – 1.378)	0.428
CANCER	104	0.986 (0.858 - 1.132)	0.814	1.003 (0.678 – 1.483)	0.991	0.807 (0.534 – 1.219)	0.304
OCULAR	75	1.049 (1.020-1.079)	0.024	1.483 (0.912 - 2.410)	0.105	1.937 (1.215 - 3.088)	0.006

Hazard Ratios and 95% Confidence Intervals for specified outcomes by randomised option, ever/never and current/not current use of tamoxifen, using the proportional hazards method

Refer to *Chapter 7, Section 7.1* for discussion of results of randomised comparison

6.4.3 Effect of age

In order to determine the effect of age on the endpoints being studied, the three analyses were repeated for all the failure events, using age at randomisation as the first covariate in the model and then extending the model, by adding either option or one of the time-dependent covariates ('Ever'/'Never' or 'Current'/'Not current' use of tamoxifen), as appropriate for the particular analysis. The results are tabulated in *Table 6.8* and the p value is that derived from the difference between the values of (-2 Log L) for the model containing only age and the extended model, using the chi-squared test statistic with one degree of freedom.

The results indicate a significantly increased incidence of all the failure events studied, except myocardial infarction and thromboembolism, with increasing age. However, the results are essentially similar to those obtained when age is not included in the model. There is some strengthening in the level of significance seen in the analysis by randomised option.

Table 6.8

	N	AGE		RANDOMISED		EVER V NEVER		CURRENT V NOT CURRENT	
		HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
MI	49	1.044 (1.013 - 1.076)	0.004	1.133 (1.089 -1.179)	<0.001	1.043 (0.358 – 1.104)	0.106	0.514 (0.269 -0.982)	0.036
IHD	67	1.036 (1.010 - 1.063)	0.004	0.903 (0.559 - 1.460)	0.671	0.836 (0.558 - 1.356)	0.470	0.753 (0.444 - 1.277)	0.285
THROMB	28	1.021(0.983-1.060)	0.267	1.097 (1.042 – 1.156)	0.008	1.859 (0.814 – 4.243)	0.126	2.229 (1.040 – 4.775)	0.038
CVA	62	1.090 (1.058-1.124)	<0.001	1.246 (1.172 – 1.325)	<0.001	2.204 (1.215 – 3.997)	0.006	0.880 (0.504 – 1.539)	0.652
FRACTURE	64	1.106 (1.072-1.141)	<0.001	0.789 (0.483 – 1.289)	0.344	0.866 (0.528-1.420)	0.569	0.805 (0.472 - 1.371)	0.419
CANCER	104	1.045(1.024-1.067)	<0.001	1.028(0.881-1.200)	0.773	0.990(0.671-1.461)	0.959	0.795(0.527-1.199)	0.280
OCULAR	75	1.076 (1.048 - 1.105)	<0.001	1.144 (1.100 - 1.190)	<0.001	1.559 (0.958 - 2.537)	0.067	1.967 (1.234 - 3.137)	0.005

Hazard Ratios and 95% Confidence Intervals for specified outcomes by adding randomised option, ever/never and current/not current use of tamoxifen to a model containing age as a continuous variable, using the proportional hazards method

Refer to Chapter 7, Section 7.1 for discussion of results of randomised comparison

6.5 Other failure events

6.5.1 Endometrial cancer

There were 11 diagnoses of endometrial cancer within the study, 3 in the observation arm and 8 in the adjuvant tamoxifen arm. Two patients (1 adjuvant, 1 observation) were recorded as having a previous uterine cancer and 1 patient randomised to adjuvant tamoxifen had metastatic disease recorded one day after the date of diagnosis of endometrial cancer. In these 3 cases, in the absence of histological confirmation, there must be some doubt as to whether they were truly new primary malignancies. Of the 8 patients in the adjuvant arm, 5 were still receiving tamoxifen therapy when their endometrial cancer was diagnosed. Median exposure of the 8 patients was 93 months. Tamoxifen status, duration of exposure and time since stopped treatment are shown in *Table 6.9*.

Table 6.9

Randomised option	Tamoxifen status	Duration (months)	Time since stopped (months)
Observation	None	-	-
Observation	None	-	-
Observation	None	-	-
Tamoxifen	Current	131	-
Tamoxifen	Stopped	107	2
Tamoxifen	Current	75	-
Tamoxifen	Current	63	-
Tamoxifen	Stopped	56	17
Tamoxifen	Current	79	-
Tamoxifen	Stopped	144	1
Tamoxifen	Current	139	-

Tamoxifen status, duration of exposure and time since stopped in 11 cases of endometrial cancer

6.5.2 Benign endometrial disease

Eleven cases of benign endometrial disease were ascertained. These were 3 cases of endometrial cystic hyperplasia, 2 cases of polyp of the corpus uteri and 6 cases of endometriosis. All 11 patients had exposure to tamoxifen, 2 of these were in the observation arm and being treated for recurrence. The median duration of tamoxifen therapy was 58 months with a range of 11 – 147 months.

6.6 Supplementary analyses

6.6.1 Ocular endpoints

In the analyses of ocular events without censoring at metastatic relapse, there were a total of 83 hospital admissions for ocular outcomes available for analysis (52 tamoxifen, 31 observation) The results of the 3 analyses by Cox proportional hazard method are shown in *Table 6.10* and show little difference from the results obtained where censoring was applied (75 cases) with only a slightly reduced level of statistical significance in the ‘Current’ vs ‘Not current’ analysis.

Figure 6.10

Analysis	HR	95% CI	p
Tamoxifen vs Observation	1.049	1.020 – 1.079	0.024
Ever vs Never	1.399	0.879 – 2.227	0.149
Current vs Not current	1.647	1.058 – 2.562	0.028

Results of comparison of incidence of ocular endpoints, with no censoring at metastatic relapse, between randomised groups, ‘Ever’ vs ‘Never’ and ‘Current’ vs ‘Not current’ use of tamoxifen

6.6.2 Fractures

The additional analyses carried out for the incidence of fractures at any site, where censoring was not applied at metastatic relapse, included a total of 86 cases of fracture (41 tamoxifen, 45 observation). This compares with 64 cases in the censored analysis. The inclusion of the presence or absence of metastases, as a time-dependent covariate, shows a strongly significant association between the presence of metastases and the incidence of fractures (HR 5.099, 95% CI: 3.132 – 9.300, $p<0.001$). The results of extending this model to compare the incidence of fracture in those randomised to tamoxifen compared with the observation group, ‘Ever’ vs ‘Never’ use of tamoxifen and ‘Current’ vs ‘Not current’ use of tamoxifen are shown in *Table 6.11*. These indicate that, when censoring at metastases is not applied, current users of tamoxifen have a significantly reduced risk of fracture compared with not current users.

Table 6.11

Analysis	HR	95% CI	p
Tamoxifen vs Observation	0.961	0.623 – 1.482	0.857
Ever vs Never	0.753	0.473 – 1.201	0.237
Current vs Not Current	0.579	0.357 – 0.939	0.022

Results of comparison of incidence of fracture between the randomised groups, ‘Ever’ vs ‘Never’ and ‘Current’ vs ‘Not current’ users of tamoxifen, adjusted for the presence or absence of metastatic disease

7.1 Statistical Aspects

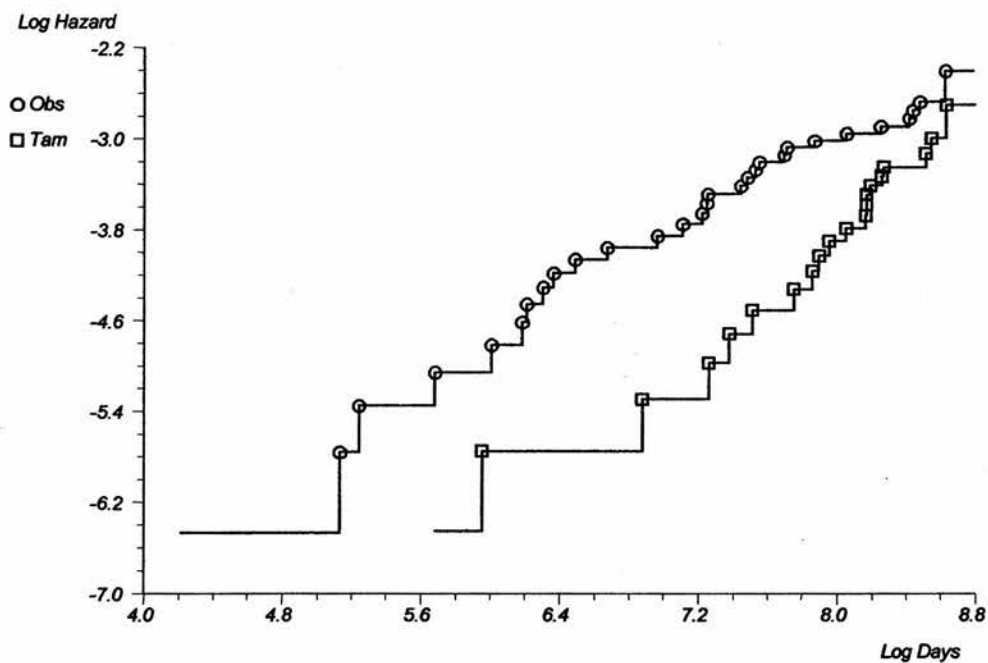
The results obtained for the analysis by randomisation, using the Cox proportional hazards model, differ substantially from the results obtained by comparing the survival curves of the two groups using the log-rank method, for the outcomes of MI, thromboembolism and CVA (*Tables 6.6 and 6.7*). There is also a difference for the ocular endpoint, although in this case the result is significant, and in the same direction, by both methods. The results for these outcomes using the proportional hazards model have hazard ratios very close to unity, with narrow confidence intervals and a statistically significant result. Interestingly, the hazard ratio for MI is highly significant ($p=0.002$) in favour of the control arm, which is in the opposite direction than is to be expected. These differences obtained by different methods suggest that the proportional hazards assumption may fail such that it is inappropriate to model the data in this way.

If the hazards are not proportional over time then the linear component of the model varies with time. This can be tested by determining the Kaplan –Meier estimate of the survivor function of the two groups. A plot of the negative logarithm of the estimated survivor function against the logarithm of the survival time (log-cumulative hazard plot) will yield parallel curves if the hazards are proportional across the two groups. The log-cumulative hazard plots for all the outcomes being studied are shown in *Figures 7.1 – 7.7*. The plots for MI and ischaemic heart disease (*Figures 7.1 and 7.2*) in particular show a tendency to converge as survival time increases, suggesting that the proportional hazard assumption is no longer valid at longer follow-up times and that the hazard associated with one or both of the groups is changing over time.

An extreme example of this is illustrated in *Figure 7.4*, where the curves for cerebrovascular disease cross and in *Figure 7.5* where the curves for new primary cancer are tending to cross towards the end of the follow-up period. . In *Figures 7.3, 7.6 and 7.7* the plots for PE/DVT, fractures and ocular disease remain essentially

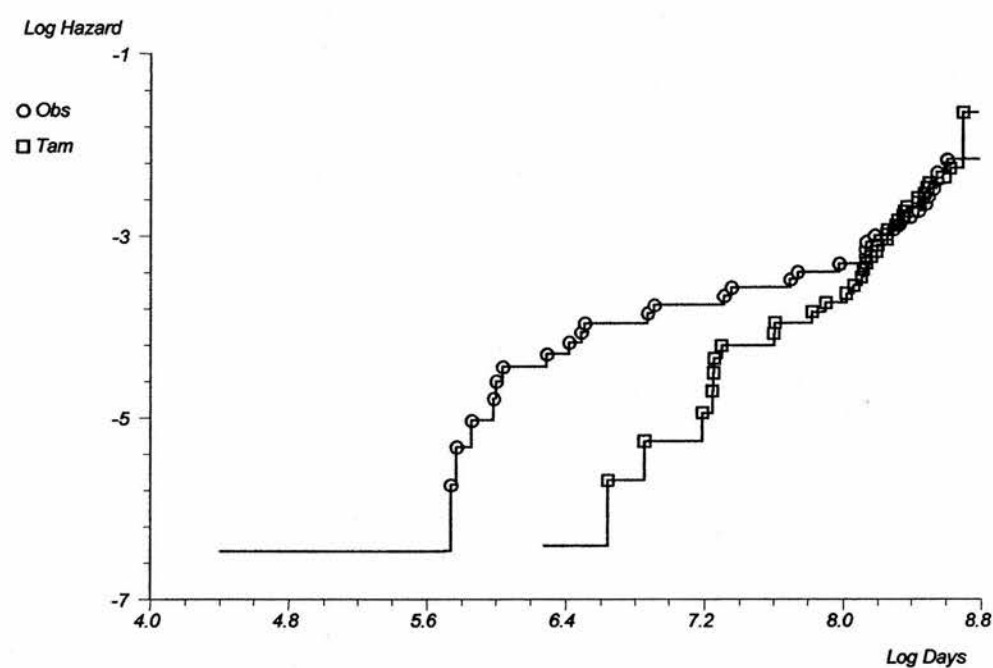
parallel throughout the follow-up period, indicating that these data fulfil the criteria for proportional hazards. Thus with some doubts as to the validity of the results of the randomised comparison by the Cox method (*Chapter 6, Tables 6.7 and 6.8*), the ‘genuine’ results of the randomised comparisons should be based on the log rank test (*Chapter 6, Table 6.6*).

Figure 7.1



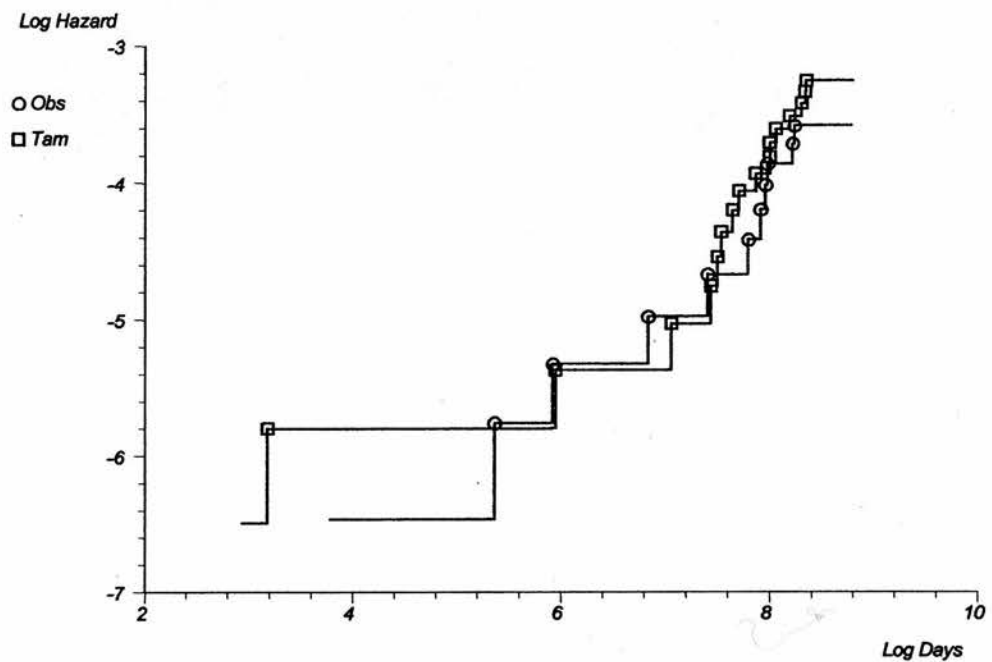
Cumulative log hazard for randomised groups with respect to MI

Figure 7.2



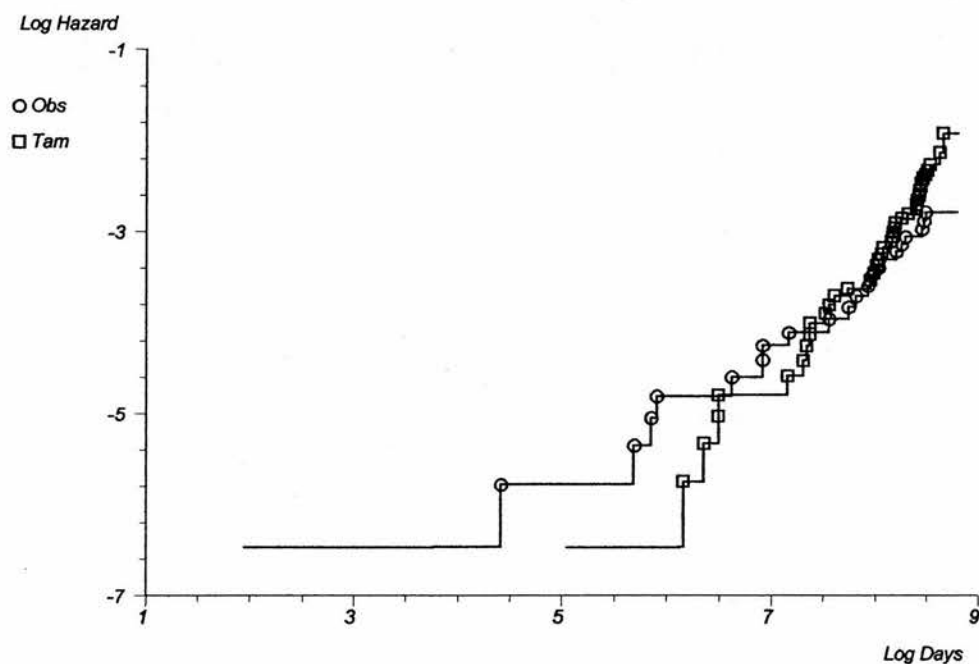
Cumulative log hazard for randomised groups with respect to other IHD (other than MI)

Figure 7.3



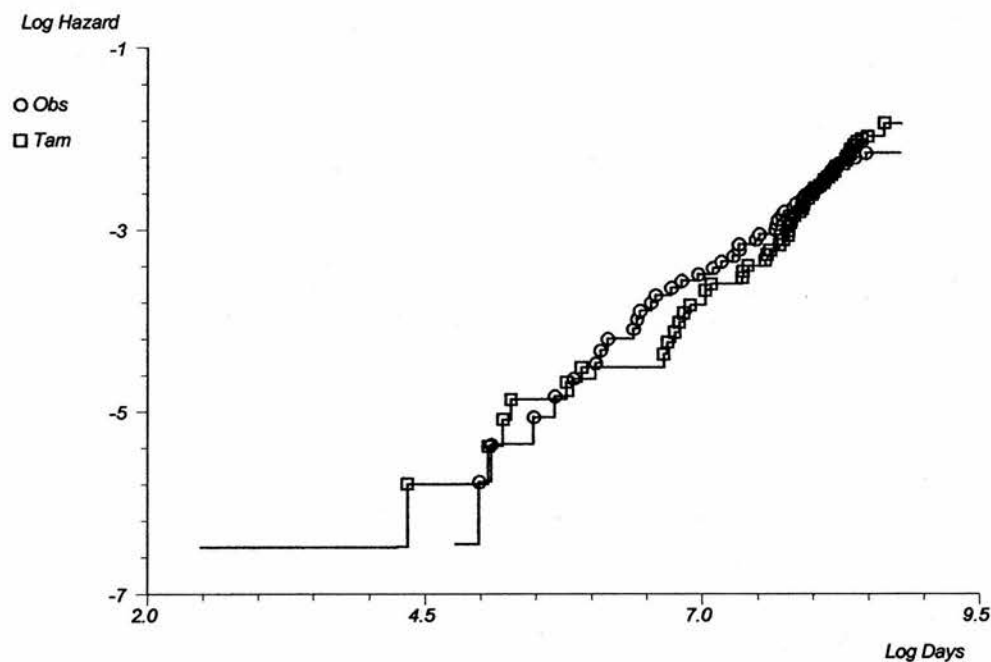
Cumulative log hazard plot for randomised groups with respect to PE/DVT

Figure 7.4



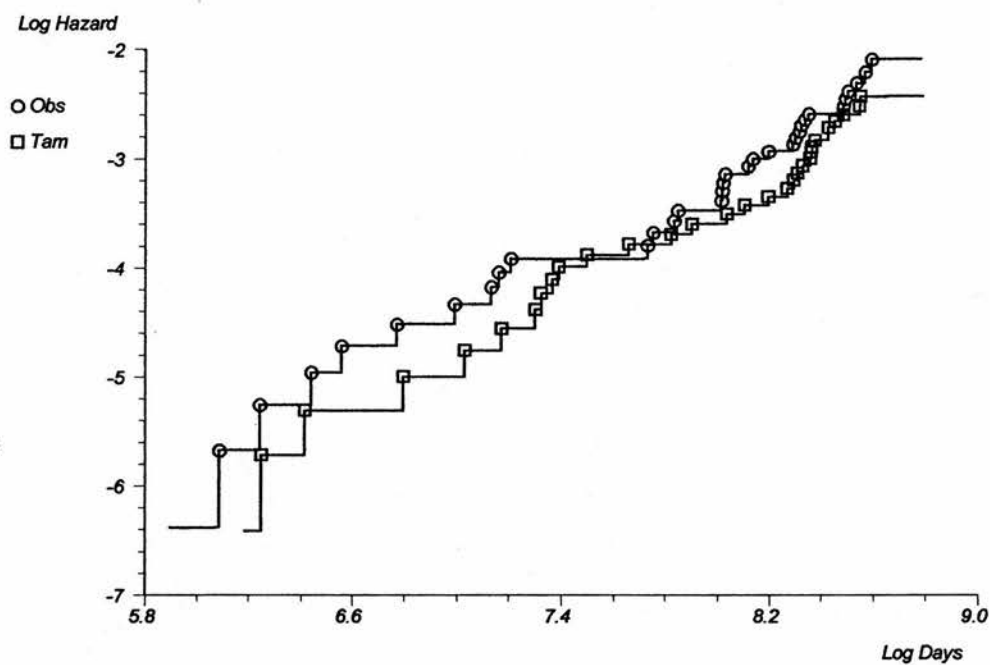
Cumulative log hazard for randomised groups with respect to CVA

Figure 7.5



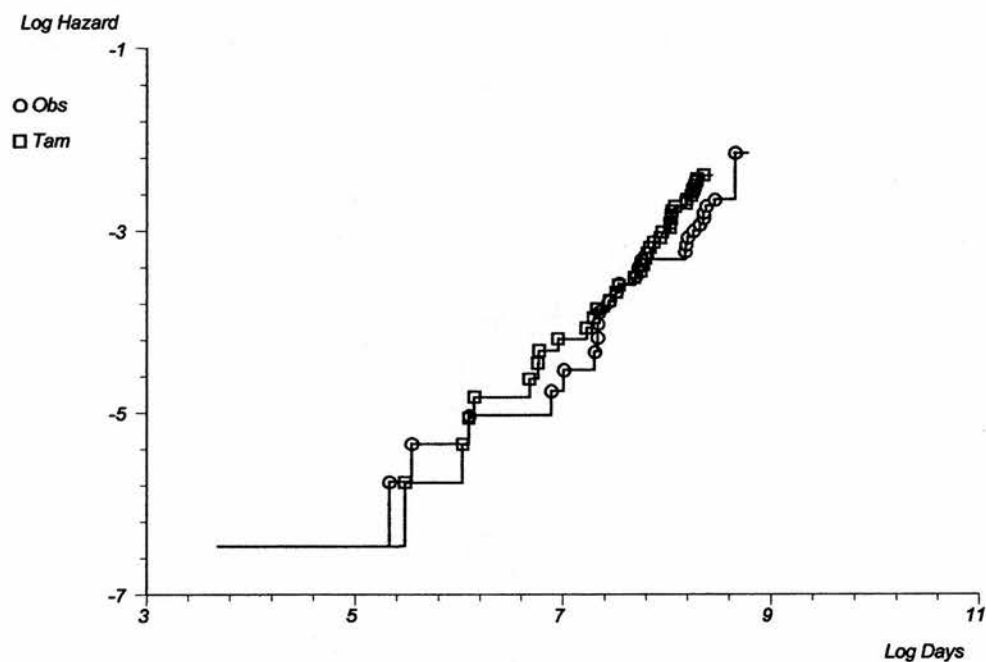
Cumulative log hazard for randomised groups with respect to new primary malignancies

Figure 7.6



Cumulative log hazard for randomised groups with respect to fractures

Figure 7.7



Cumulative log hazard for randomised groups with respect to ocular toxicity

In his original paper, Cox suggested testing the interaction between the effect of a covariate (or set of covariates) and time (Cox, 1972). If a time dependency of the effect of a covariate on risk is established, a model with time-varying coefficients can be used (Abrahamowicz et al., 1996). An alternative approach is to divide the follow-up time into intervals and determine differences between the relative risks in each interval evaluated (Moreau et al., 1985). The disadvantages of this method are that the selection of intervals is arbitrary and the assessment of whether a time dependency is present is subjective. The use of these two models to test the proportional hazards assumption in a population-based study of acute myeloid leukaemia has recently been described (Bourdais-Mannone et al., 1999).

In principle, all of the methods available for testing the assumption of proportional hazards can be applied to the situation where the value of a covariate varies over time (as in the ‘Ever/Never’ and ‘Current/Not current’ analyses discussed in *Chapter 5, Section 5.5*) (Altman & De Stavola, 1994), but they are much more complex to implement. The concept of the value of a covariate varying over time should not be confused with that of the value of a coefficient (i.e. the effect on risk) varying over time. Both are often referred to as time-dependent covariates. Despite all the methodologies available for model checking, the overall power to detect departures from the proportional hazards assumption is generally low because of the semi-parametric nature of the model. Investigators are often reluctant to test the assumption because of the difficulty in estimating and interpreting the effect of a predictor once the hypothesis of proportional hazards has been rejected.

Use of the proportional hazards model in situations where the impact of a variable may change during follow-up can introduce a bias in the estimation of the predictor’s effect. Further, if the effect of the variable inverts during the course of follow-up, a false conclusion of no difference between the groups may be made. The use of other

statistical methods, such as non-parametric survival analysis methodology) was beyond the scope of this dissertation. This said, it is important that the possible limitations that have been identified in the validity of the model should be borne in mind when considering and interpreting the results of the study. Some of these alternative approaches may be considered relevant for future work, particularly if a reanalysis of these data, with further follow-up, is planned.

7.2 Risk/Benefit assessment of tamoxifen

The benefit of tamoxifen treatment for patients with breast cancer is now undisputed. Current studies are addressing the issues of optimal duration of treatment, combining tamoxifen therapy with chemotherapy or other hormone therapies and the effectiveness of tamoxifen in the chemoprevention of breast cancer. Long-term morbidity is particularly important in the context of extended duration of treatment and the medication of young, healthy women considered to be at high risk of developing breast cancer. Long-term morbidity is however, as yet, poorly described with judgements to date being based on relatively short durations of treatment, of up to 5 years. This study provides important data relating to key outcomes in the study of tamoxifen morbidity in women who have received up to 18 years of treatment and therefore serves to fill a gap in our current knowledge.

The additional follow-up in this study (01/01/93 – 31/03/96) compared to the previous (*Appendix 2*) has provided an additional 12 cases of myocardial infarction, 25 cases of other ischaemic heart disease, 17 cases of cerebrovascular disease and 3 cases of thromboembolism. The results obtained for the individual outcomes studied are discussed below.

7.3 Myocardial infarction and ischaemic heart disease

The cardioprotective action of tamoxifen is undoubtedly a key element in discussing the likely risk to benefit ratio of tamoxifen treatment and has probably received the greatest attention in the medical literature. The results of the current study suggest that this effect is limited to a reduction in the incidence of acute myocardial infarction in current tamoxifen users compared with previous or non-users (HR=0.511, 95% CI: 0.267 - 0.976, $p=0.034$). Thus the previous results obtained for the 'Current/Not current' comparison are confirmed with an additional 3 years and 3 months of follow-up while the results for the 'Ever/Never' comparison are no longer statistically significant. The introduction of age into the model indicates that, as would be expected, the incidence of all ischaemic heart disease increases with increasing age but the effect of tamoxifen does not change when age is taken into account.

The results obtained suggest that the protective effect of tamoxifen is only apparent while receiving treatment and is lost on the cessation of treatment. This concurs with findings that the beneficial effects of tamoxifen on lipid profile are not maintained after cessation of treatment (Dewar et al., 1992) (Cuzick et al., 1993). The interaction of tamoxifen with membranes and lipoprotein particles and the resulting antioxidant and cardioprotective action is also unlikely to be maintained once exposure to tamoxifen has been stopped.

There is no evidence from the current study that tamoxifen therapy protects against ischaemic heart disease other than MI, such as coronary failure, coronary insufficiency, angina pectoris or atherosclerotic heart disease.

Two large clinical trials of adjuvant tamoxifen therapy have reported results for cardiac mortality and morbidity. The Stockholm adjuvant tamoxifen trial looked at the incidence of hospital admissions due to cardiac disease and the relative hazard for those randomised to receive tamoxifen compared with the control arm, at a median

follow-up of 6 years, was 0.68 (95% CI: 0.48 – 0.97, $p=0.03$). There was also a statistically significant difference in the comparison of 2 years of tamoxifen versus 5 years of tamoxifen (relative hazard 0.37, 95% CI: 0.15 – 0.92, $p=0.03$). Cardiac disease in this study included myocardial infarction, other types of ischaemic heart disease and miscellaneous cardiac disease. There was no significant difference between the treatment arms for the subgroup of myocardial infarction (Rutqvist & Mattson, 1993).

The NSABP-B14 study reported a non-significant reduction in cardiac mortality for tamoxifen-treated patients (8 deaths) compared with those receiving placebo (12 deaths) with a relative hazard of 0.66 (95% CI: 0.27 – 1.61). The mean follow-up time was 8.9 years. When possible coronary deaths were included in the analysis, the relative hazard was 0.85 (95% CI: 0.46 – 1.58) (Constantino et al. 1997). The analyses for both the Stockholm and NSABP B-14 analyses were on an ‘intent-to-treat’ (ITT) basis, comparing the groups as they were randomised regardless of whether the treatment was received. This tends to provide a more conservative estimate of risk and biases the result towards the null hypothesis, especially if the hypothetical benefit is for current use of tamoxifen.

Other studies have failed to show an effect of tamoxifen on cardiac morbidity and mortality. The results from the 1995 meta analysis by the EBCTCG showed no difference in the cardiac mortality in the tamoxifen treated patients compared with controls (Early Breast Cancer Trialists' Collaborative Group, 1998) and there was no difference in the rate of ischaemic heart disease between the tamoxifen and placebo groups in the US breast cancer prevention trial (BCP Trial) (Fisher et al., 1998).

Coronary heart disease in premenopausal women is rare and there is no evidence from this or any other published study to date that tamoxifen increases the risk of cardiac morbidity in younger women. This is an important observation with regard to the use of tamoxifen as a prophylactic in healthy, premenopausal women. One of the postulated mechanisms by which tamoxifen exerts a cardioprotective effect is by an action on arterial status similar to that exerted by oestrogen. Oestrogen is known to act as an arterial vasodilator and decrease the pulsatility index (PI), which is a measure of impedance to blood flow within the artery. This has been demonstrated in uterine and cerebral arteries in women (Penotti et al., 1998) and the action is the expression of a generalised effect on arterial vessels. However studies have shown that tamoxifen does not exhibit this effect and this has implications with regard to the cardioprotective actions of tamoxifen. The authors suggest that its use in healthy women should therefore be reviewed.

7.4 Thromboembolic disease

The results of the current study with regard to thromboembolic disease again reinforce those obtained in the previous study of morbidity within the Scottish adjuvant tamoxifen trial. They suggest that patients currently receiving tamoxifen are more than twice as likely to suffer an incident of pulmonary embolism or deep vein thrombosis than those who have stopped, or never received, treatment (HR 2.229, 95% CI: 1.040 – 4.777, $p=0.038$). In the BCP Trial there is also evidence of an association of tamoxifen treatment with an increased risk of thromboembolism. Seventeen cases of pulmonary embolism occurred in the tamoxifen arm compared with 6 on placebo and there were 30 cases of deep vein thrombosis in the tamoxifen group compared with 19 in the placebo group (Fisher et al., 1998).

The Stockholm adjuvant tamoxifen trial was unable to show a difference in the incidence of hospital admission for thromboembolic causes between those randomised

to tamoxifen and the control group (Relative Hazard 1.06, 95% CI: 0.71-1.60). This study included cerebral thrombosis in the thromboembolic group, differential diagnosis between thrombotic and haemorrhagic CVAs being performed routinely in Sweden on admission to hospital. As discussed in relation to cardiac morbidity, the ITT analysis used in this study is likely to be less able to detect a difference between the groups, particularly if current use is an important factor.

The mechanism of a possible effect of tamoxifen on thromboembolism is not clear and is likely to be multifactorial. Changes in coagulation factors which have been noted on tamoxifen therapy, such as a decrease in levels of antithrombin III, are small and unlikely on their own to account for the results observed. Activated protein C resistance due to Factor V Leiden is a significant inherited prothrombotic defect in European populations. A case-report has identified 3 cases of thromboembolic complications in women receiving tamoxifen therapy all of whom were determined to be heterozygous for Factor V Leiden. The authors suggest that patients should be screened for Factor V Leiden before commencing tamoxifen therapy (Weitz et al., 1997).

7.5 Cerebrovascular disease

The results obtained for the incidence of cerebrovascular disease indicate a significant increase in the incidence of CVA in those patients who have ever received tamoxifen compared with never users. The 'Current' versus 'Not current' comparison indicates no difference between the groups indicating that, for incidence of CVA, it is ever use of tamoxifen rather than current use which may be important. These results differ from those obtained in the previous study, where no significant differences were observed in any of the analyses performed, with respect to CVA. This may be a reflection of the increased follow-up time and increased number of events (from 45 to 62). It should be remembered that the curves in the log-cumulative hazard plot for the

randomised comparison crossed towards the end of the follow-up period, indicating that these results may not be reliable, particularly towards the end of the follow-up period (*Figure 7.4*).

Cerebrovascular disease includes both thrombotic and haemorrhagic events and current tamoxifen use does appear to increase the risk of thrombotic events (*Section 7.4*). The increased incidence of CVA in patients who have received tamoxifen may therefore be a reflection of an increased number of thrombotic events in these patients. Of the cerebrovascular events identifiable by ICD9 code as either as haemorrhagic (430, 431, 432) or thrombotic (433, 434), there were 5 in the former group and 10 in the latter group. However, the majority of cerebrovascular events are classified as ‘ill-defined’ and no conclusions can be drawn about their thrombotic or haemorrhagic nature. The BCP Trial reported an increased rate of stroke in the tamoxifen group (38 events) compared with placebo (24 events), although this was not significant (RR 1.59, 95% CI: 0.93-2.77) (Fisher et al., 1998).

7.6 New primary malignancies

The carcinogenic potential of tamoxifen is particularly important in view of the very widespread use of tamoxifen in women with a high cure rate and long life expectancy, and in healthy women in the context of breast cancer chemoprevention. A hepatocarcinogenic effect has been clearly demonstrated in rats (*Chapter 3, Section 3.3.3*). In fact, only one case of liver cancer was identified in the current study, in a patient who had received tamoxifen therapy for 66 months. This low incidence was in spite of the extended duration of exposure and supports the reassuring data obtained from other studies. One reason for the apparently low incidence of primary liver tumours may be that histology is rarely available to allow the differential diagnosis of primary tumour as opposed to metastases from breast carcinoma to be made. Overall

in the current study, there was no significant difference in the total number of new primary malignancies by any of the analyses performed.

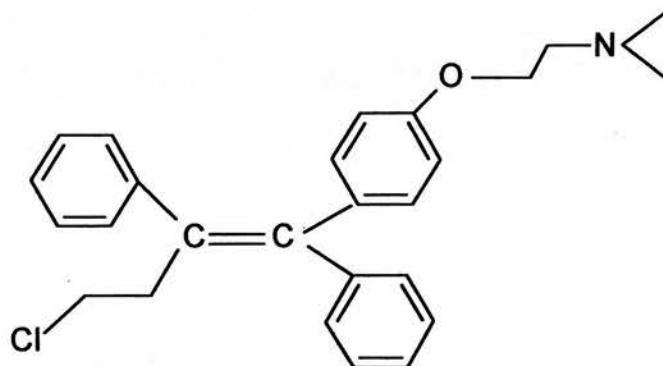
The number of endometrial cancers detected was higher in the tamoxifen arm than in the observation arm but the number of cases overall was low and insufficient for statistical analysis, reflecting the fact that this is a rare condition. Nevertheless, the results offer limited support for the considerable volume of data already available indicating that women on long-term tamoxifen treatment are at a higher risk of developing endometrial cancer. In the BCP Trial, thirty-three (33) women on tamoxifen developed endometrial cancer compared with 14 women on placebo. The relatively rare incidence of endometrial carcinoma must, however, be put in perspective in relation to hundreds of thousands of women who have benefited from tamoxifen treatment.

One of the criteria in developing new endocrine treatments for breast cancer is to find an effective treatment that does not exhibit the carcinogenic effects of tamoxifen. Toremifene is a chlorinated derivative of tamoxifen (*Figure 7.8*) and is marketed as Fareston® for the treatment of metastatic breast cancer. A number of preclinical studies have shown that toremifene does not appear to have the same carcinogenic potential as tamoxifen. A comparison of the hepatoproliferative effects of tamoxifen with toremifene has been investigated in rats using a ^{32}P -postlabelling assay. Administration of tamoxifen at 45 mg/kg for 7 days produced liver DNA nucleoside modifications, but no detectable modified bases in rat liver DNA were produced by toremifene (Hard et al., 1993). Also, unlike tamoxifen, toremifene does not show activity in the rapid induction of hepatocellular altered foci (HAF) in rats (Williams et al., 1997). To further evaluate toremifene for possible genotoxicity, 3 standard in vitro assays were performed in which toremifene failed to exhibit genotoxicity or myelotoxicity. However the action of toremifene on the endometrium in an athymic

ovariectomised mouse model is identical to that of tamoxifen. In contrast, the steroidal antioestrogen

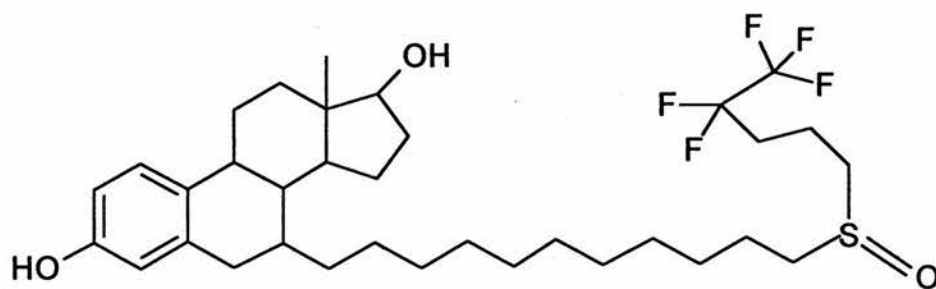
ICI 182780 (*Figure 7.9*) inhibited tamoxifen stimulated endometrial cancer, both in the presence and absence of oestrogen, in this model (O'Regan, et al., 1998). ICI 182780 is a 'pure' antioestrogen and as such, does not exhibit any oestrogen agonist activity. To date, the clinical use of toremifene has been in the treatment of advanced breast cancer and there are few clinical data on its use in the adjuvant setting. A large ongoing study being co-ordinated by the Finnish Breast Cancer Group is comparing toremifene and tamoxifen as adjuvant therapies in breast cancer patients. An interim safety analysis on 500 patients with a mean follow-up of 18 months has shown no differences between tamoxifen and toremifene in terms of efficacy or side effects (Holli, 1998).

Figure 7.8



Structure of toremifene

Figure 7.9



Structure of ICI 182780

7.7 Effects on the skeleton

The results of the randomised, 'Ever/Never' and 'Current/Not current' comparisons, where censoring has been applied at metastatic relapse, indicate a similar rate of fracture incidence in the groups being compared. Thus, there is no indication from these results that tamoxifen has an effect on the pathogenesis of fracture in patients with breast cancer. In the supplementary analyses, in which censoring at metastatic relapse was not applied, there is a clear and not surprising association between the presence of metastatic disease and the incidence of fracture ($p < 0.001$). In this analysis, a significant reduction in fractures in current users of tamoxifen compared with not current users is also seen ($p = 0.022$). This observation could be related to a reduced incidence of metastatic disease in current users of tamoxifen.

One other study has reported on the occurrence of fracture in breast cancer patients receiving tamoxifen. Linkage of data, pertaining to patients randomised within a trial of adjuvant tamoxifen in breast cancer run by the Danish Breast Cancer Co-operative Group, with the Danish National Registry of Patients indicates that there is no significant difference in the incidence of femoral fracture between the two groups (64 in the tamoxifen treated group and 51 in the control group, HR = 1.08, 95% CI: 0.74 – 1.55, $p = 0.70$). There was a slight excess of trochanteric fractures in the tamoxifen group (27) compared with controls (11) and this was statistically significant (HR 2.12, 95% CI: 1.12 – 4.01, $p = 0.022$) (Kristensen et al., 1996). These results could not be explained by a longer survival in the tamoxifen group nor by the presence of bone metastases resulting in pathological fractures.

These results differ from those obtained in the BCP Trial where a clear reduction in hip, radius (Colles') and spine fractures associated with tamoxifen administration is evident. There were a total of 71 fractures in the placebo group compared with 47 in the tamoxifen arm (Fisher et al., 1998). This effect of tamoxifen in healthy women concurs with evidence of a protective effect in healthy women of exogenous oestrogens on bone density and a decreased fracture risk. This has been shown for postmenopausal oestrogen use (Christiansen & Lindsay, 1990) (Michaelsson et al., 1998). The results of a Swedish case-control study in 130 cases and 562 controls suggest that OC use late in reproductive life may also confer a reduction in the risk of hip fracture (Michaelsson et al., 1999).

Thus, there is therefore some evidence of an oestrogen-mediated reduction in fractures in healthy women and effects are seen in women with breast cancer which depend on the fracture site. These various findings are difficult to interpret and the results obtained are likely to be influenced by a number of factors. For example, there are data which show a 5 times increased risk of vertebral fracture in patients with breast cancer compared with healthy controls (OR = 4.7, 95% CI: 2.3 – 9.9), suggesting that the presence of breast cancer itself can cause changes in skeletal metabolism (Kanis et al., 1999). This concurs with data showing a decrease in bone mineral density in postmenopausal women with breast cancer compared to normal women ($p < 0.001$) (Kostoglou-Athanassiou et al., 1994). There are also apparent differences in the effect of tamoxifen on the skeleton which depend on the hormonal environment. In the Royal Marsden pilot chemoprevention trial, postmenopausal women receiving tamoxifen showed a mean annual increase in BMD of 1.17% in the spine and 1.71% in the hip compared with a non-significant loss for women on placebo. However, in premenopausal women receiving tamoxifen there was a mean annual loss of BMD in the spine of 1.44% compared with a small annual gain (0.24%) for the placebo group (Powles et al., 1996).

The answer to the exact actions of tamoxifen on the skeleton may be found at the level of the bone cells. As yet, the effect of tamoxifen on osteoclasts and osteoblasts is largely unknown but the oestrogenic effect may be dependent on the presence of oestrogen receptors on the cells (Ernst et al., 1991). Oestrogen receptors have been detected in some osteoblastic cell lines, usually in low concentrations (Keeting et al., 1991). It is therefore clear that an effect of tamoxifen therapy on fracture incidence has not been clearly established and further prospective studies are required before any firm conclusions can be drawn.

7.8 Ocular effects

The current study provides evidence of a very significant association between tamoxifen use and hospitalisation for ocular problems, both in the comparison of the randomised groups and the ‘Current’ ‘Not current’ use groups. This effect is independent of the effect of age, which itself is highly significant. The majority of the episodes of hospitalisation identified were for cataract (59 out of 96 events). Almost twice as many cataracts were recorded in patients randomised to receive tamoxifen compared with those in the observation arm. The results did not change materially when the data were re-analysed without censoring at metastatic relapse.

Cataract is the commonest cause of visual impairment in persons aged 75 years or older. In a population-based study of the incidence of age-related cataracts in 3,271 participants aged from 40 to 98 years, the overall prevalence of any type of cataract that had not been surgically corrected was 18% (McCarty et al., 1999). Another population based study collected data between 1988 and 1995 and reported a cumulative incidence of nuclear cataract, in persons aged 75 years or older, of 40% with women more likely than men to be affected, after adjusting for age (Klein et al., 1998). Further, the Rotterdam Study has examined the prevalence and causes of blindness and visual impairment in 6,775 subjects and concluded that age-related

cataract is primarily responsible for the increased prevalence of visual impairment (Klaver et al., 1998).

The results of the current study concur with those obtained in the TOES study (*Chapter 3, Section 3.5.2*) in which the ‘previous tamoxifen use’ group had a significantly increased incidence of posterior subcapsular opacities (OR = 4.02, $p=0.046$) compared with untreated controls. There was also an increased incidence in the ‘current tamoxifen use’ group which failed to reach statistical significance (OR = 4.07, $p=0.067$). The similar prevalence of posterior subcapsular opacities in the two tamoxifen treatment groups suggest that any effect is occurring within the first 5 years of drug exposure. In contrast, the survival curves for ocular outcomes in the current study suggest that separation between the two groups is not occurring until about Year 8 (*Figure 6.7*). The BCP Trial has also reported a significant increase in cataract in the tamoxifen group. The development of cataracts since randomisation relied on self reporting and was marginally statistically significant (RR 1.14, 95% CI:1.01-1.29). However, the incidence of cataract surgery was verified and documented by examination of medical records. There was an increased risk of developing cataract and undergoing surgery in the tamoxifen group compared with the placebo group (RR 1.57, 95% CI: 1.16-2.14) (Fisher et al., 1998).

The observed clinical results support the observations made in tissue culture experiments in bovine lenses (*Chapter 3, Section 3.5.*). Similar experiments using the steroidal antioestrogen (ICI 182780) (*Figure 7.2*), which is a pure antioestrogen with no oestrogenic effects, showed that this did not block volume-regulated chloride currents in three cultured cell lines and required 10-fold higher concentration to induce significant opacification of bovine lenses in vitro (Zhang et al., 1995). Studies in rats have shown that treatment with tamoxifen for 2 years results in increased in cataract formation (Greaves et al., 1993). There is also some evidence that postmenopausal oestrogen replacement therapy can increase the prevalence of

posterior subcapsular cataracts. The Blue Mountains Eye Study has shown that the prevalence of posterior subcapsular cataract is increased in current users of hormone replacement therapy who had had a non-surgical menopause (adjusted OR = 2.1 (95% CI: 1.1 – 4.1) (Cumming & Mitchell, 1997).

Other factors which may predispose to the formation of cataracts include long-term corticosteroid use and the presence of diabetes mellitus. It is not possible in this study to adjust for these factors although they would be expected to be evenly distributed within the two randomised groups and therefore not affect the randomised comparison, which also showed a borderline significant increase of all ocular toxicities ($p=0.049$).

It should be remembered that the cases of cataract determined in the study were all related to an episode of inpatient hospitalisation and were likely to be cases undergoing surgery for removal of the lens and implantation of an artificial intraocular lens. The association between tamoxifen therapy and the rate of hospitalisation due to cataract observed in the current study, could potentially have implications for the provision of ophthalmic services and future requirements for cataract surgery.

Screening for ocular toxicity

Based on the current evidence available on the incidence of ocular toxicity associated with tamoxifen use, views on whether screening for ocular disorders is merited in women receiving tamoxifen therapy are mixed. Extensive ocular examination of 135 visually asymptomatic tamoxifen treated patients with a mean cumulative dose of 17.2 g, revealed two patients with intraretinal refractile crystals. The authors conclude that the relatively uncommon finding of ocular toxicity does not merit special screening (Heier et al., 1994). This view was supported in a study of retinal changes and visual impairment in 290 patients taking tamoxifen for durations of between 6 months and 12 years (Tang-R et al., 1997). A study of 61 patients found 2 with retinopathy, one

with corneal deposits and one with optic neuritis. Based on these results the author recommends an ophthalmological assessment in the case of visual complaints for those patients taking tamoxifen (Therssen et al., 1995). The same conclusion was drawn following a review of the medical literature published in 1994 where case reports and clinical studies citing adverse ocular effects in tamoxifen treated patients were identified (Heier et al., 1994). Routine ophthalmological screening examinations are considered as being of potential benefit because many of the lesions identified are reversible on the cessation of treatment (Ah-Song & Sasco, 1997). The conclusion drawn from the TOES study is that women should have a thorough baseline ophthalmic evaluation within the first year of starting treatment and subsequently receive appropriate follow-up evaluations (Gorin et al., 1998).

7.9 Benign endometrial disease

This study provided little evidence of an effect of tamoxifen therapy on the development of benign endometrial lesions. This is not surprising when it is considered that the majority of benign endometrial changes reported elsewhere have been detected in asymptomatic women. Transvaginal ultrasonography is used to measure endometrial thickness and in the most recent results from the Royal Marsden pilot chemoprevention study a persistent endometrial thickening of 8 mm or more was identified in 56 (24%) of the 235 women on tamoxifen compared with only 5 (2%) of 228 women on placebo (Powles et al., 1998). This endometrial thickening, and a consequent risk of developing endometrial cancer, was limited to postmenopausal women and recently amenorrheic women with a low level of circulating oestradiol. The results lead the authors to conclude that women who develop amenorrhea on tamoxifen, especially in the presence of endometrial thickening, low oestradiol levels with or without gynaecological symptoms warrant further investigation (Chang et al., 1998).

There has been much controversy and lively discussion about the level of monitoring required of women on long-term tamoxifen therapy. It is not possible to predict which postmenopausal women will develop pathological endometrial changes and some have suggested routine periodic endometrial sampling for patients on tamoxifen (Cohen et al., 1993) or regular monitoring by less invasive means, such as transvaginal ultrasonography (Kedar, 1994). However, other groups do not agree with this approach and the issue is still a controversial one (Bisset, 1994, Seoud et al., 1994). A study at the Edinburgh Breast Unit investigated endometrial thickness in 487 women with breast cancer. Three hundred and fifty seven (357) women treated with tamoxifen for between 5 and 191 months had significantly thickened endometria measured by transvaginal ultrasound compared with 130 controls ($p < 0.0001$). Further assessment of thickened endometria by hysteroscopy revealed a 46% false positive scan rate suggesting that this method of screening in asymptomatic women is unjustified (Love et al., 1999).

7.10 Dementias

This study was unable to demonstrate any association between tamoxifen use and the incidence of dementia in the 18 cases that were detected. There is, as yet, no clear unambiguous evidence of the benefit of oestrogen, although there are plausible biological mechanisms by which oestrogen might lead to improved cognition and reduced demential risk. These include the promotion of cholinergic and serotonergic activity in specific brain regions, maintenance of the network of neurones, favourable effects on lipoprotein profile and prevention of cerebral ischaemia. Meta analyses of 10 observational studies suggest a 29% decreased risk of developing dementia among oestrogen users but the findings of the studies are heterogenous. Thirteen studies addressing the effects of oestrogen on cognitive function have failed to show a clear benefit. Studies of oestrogen therapy in Alzheimer's disease have generally had positive results but have been small trials of short duration, non-randomised and uncontrolled (Yaffe et al., 1998). The results of a three year, multicentre, double blind,

placebo controlled trial of various oestrogen/progestin interventions in 875 postmenopausal women showed no effect on cognitive function (Greendale et al., 1998).

There are, to date, no data available describing the effect of tamoxifen on cognitive function or incidence or severity of dementia. Large placebo-controlled trials are required to determine whether tamoxifen has a role to play in this area.

7.11 The tamoxifen life-cycle

The original intended use of tamoxifen was as a ‘morning-after pill’ for contraception but this was shown to be inappropriate. Now tamoxifen is the first line endocrine treatment of choice in women with breast cancer and its use in this indication is widespread. Early use was for the palliation advanced breast cancer and then, having been shown in numerous clinical trials to be a safe and effective therapeutic option, its use was extended to treatment in early disease. Its effectiveness in the chemoprevention of breast cancer is currently being studied and tamoxifen is now licensed in the US for this indication in women at high risk of the disease.

Table 7.1 summarises the risk to benefit profile of tamoxifen as it is currently understood from this study and those preceding it. Work is still required to clarify many of the questions for which there are, as yet, no definitive answers.

Table 7.1

Risks	Benefits
Increased incidence of PE/DVT	Increased breast cancer survival
Increased incidence of stroke	Decreased incidence of contralateral breast cancer
Increased cataract formation	Possible decreased breast cancer incidence in women at high risk
Increased endometrial cancer associated with extended duration of exposure	Decrease in MI incidence in current tamoxifen users
	Maintenance of BMD
	Possible effect on the incidence of fractures
	Possible improvement in cognitive function

Summary of risk to benefit profile of tamoxifen in healthy women and those with breast cancer

Progress in understanding the basic and clinical pharmacology of tamoxifen and its mode of action as an oestrogen agonist and antagonist, and elucidation of the cell-signaling processes involved in the interaction with the ER, have resulted in the development of a new generation of compounds with wider applications and has heralded a new era in preventative therapeutics that could revolutionize womens' health (Jordan, 1997). The nature and use of these new pharmaceuticals is discussed more fully in *Chapter 12*.

8.1 Introduction

It has long been recognised that postmenopausal hormone replacement therapy (HRT) and oral contraception (OC) are associated with a spectrum of both risks and benefits. Both therapies involve the use of exogenous oestrogen, either alone or in combination with progestagens. The well-documented profile of benefits associated with postmenopausal oestrogen use includes reduced cardiovascular disease and protection of the skeleton against osteoporosis. Concerns about the risks associated with exogenous oestrogen exposure have focused on areas such as thromboembolism and cancer risk. The published literature is reviewed in the following sections of this chapter, with some of the most recent published data being discussed in *Chapter 11*.

Many of the most common cancers occur in sites that are under hormonal regulation by the steroid sex hormones. For women, these include the breast, ovary, endometrium and, possibly, the colon. The use of oestrogens and progestagens for contraception and postmenopausal hormone replacement therapy has provided indirect information on the effect of exogenous hormone exposure on the incidence of these cancers.

The evidence of cancer risk associated with exposure to exogenous oestrogens is conflicting, with apparently increased risk at some sites and protection at others. The risk appears to differ according to menstrual status and levels of endogenous oestrogens. The most intensive debate on the possible increased cancer incidence associated with exogenous oestrogen exposure as HRT or OC has always centered around the risk of breast cancer. Small studies often show no effect but they have low statistical power and are therefore unreliable. For example, no increase in the incidence of breast cancer was observed in 142 women receiving combined OC for 7 years, compared with controls. The same study showed no increase in breast cancer

incidence in 98 postmenopausal women who received HRT with oestrogens and progestagens for the same period of time (Tzingounis et al., 1996).

The preference of researchers is to perform large-scale epidemiological studies, involving many thousands of women and long-term follow-up. Meta analyses of published data are also used in an attempt to quantify the risks and benefits associated with oestrogen use. Duration of oestrogen exposure, whether exposure is current or previous and time since last exposure are important variables to be taken into account as part of these investigations. Some of the epidemiological studies that have been carried out in this field are described below, and the results are discussed in the relevant sections that follow.

8.2 Epidemiological studies of exogenous oestrogen use

8.2.1 The Nurses' Health Study

The Nurses' Health Study is a large prospective cohort study initiated in the US in 1976. 121,700 female registered nurses of between 30 and 55 years of age completed a mailed questionnaire concerning their medical history. This included information on oral contraceptive use, menopause, cardiovascular disease and cancer. Follow-up is biennial, by mailed questionnaire, at which time further information on exposures and newly identified cases of major illness is sought. The study has maintained a high follow-up rate, with >90% of participants responding to each of the follow-up cycles between 1988 and 1997. More recently the study has been extended to address other emerging issues in women's health (Colditz et al., 1997).

8.2.2 Royal College of General Practitioners' Study

The Royal College of General Practitioners initiated a cohort study in the UK in 1968 into which 23,000 women taking OC and 23,000 women who had never used them were recruited. Details of morbidity and mortality are reported every six months by

general practitioners and the study had 25 years of follow-up when reported in 1999. The primary aim of the study was to describe the long-term effects of OC use on mortality (Beral et al., 1999). Over the 25 year follow-up period, 1599 deaths have been reported. For women who had stopped OC use 10 years or more previously, there were no significant excesses or deficits, either overall or for any specific cause of death. Among current and recent (within 10 years) users, a decrease in the relative risk of death from ovarian cancer has been reported along with increased risk of death from cervical cancer, likely to be attributable to absence of barrier contraception use, and cerebrovascular disease.

8.2.3 World Health Organisation Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception

This hospital based case-control study was undertaken in 21 centres in 17 countries. Cases and up to three controls, matched by 5-year age band, were interviewed while in hospital with a standard questionnaire, which included information on medical and personal history and lifetime contraceptive use. Cases were those admitted with a diagnosis of acute myocardial infarction, pulmonary embolism or deep vein thrombosis between February 1989 and January 1995 (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1997) (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1995).

8.3 Hormone Replacement Therapy (HRT)

8.3.1 HRT and breast cancer

The risk of breast cancer associated with postmenopausal hormone use in the Nurses' Health Study was reported in 1995 (Colditz et al., 1995). The data showed a significantly increased risk of breast cancer in women currently using oestrogen alone (RR 1.32, 95% CI: 1.14-1.54). The addition of progestins to oestrogen therapy did not

reduce the risk of developing breast cancer (RR 1.41, 95% CI:1.15-1.74). The relative risks associated with 5-9 years of exposure and 10 or more years were similar (RR=1.46), but the increased risk associated with 5 or more years of postmenopausal therapy was greater among older women (RR 1.71, 95% CI:1.34-2.18 for women of 60-64 years). The increased risk of developing breast cancer was translated into a significantly increased risk of death from breast cancer among women who had taken oestrogen therapy for five or more years (RR 1.45, 95% CI:1.01-2.09).

These data suggesting a positive relationship between the use of oestrogens after the menopause and an increased risk of breast cancer are supported by a large number of other observational studies which have been published in the literature. Duration of exposure appears to be an important factor and a reanalysis of data from 51 studies, including more than 52,000 patients with breast cancer and more than 100,000 women without breast cancer indicates that, for each year a woman uses postmenopausal hormones, her risk of breast cancer increases by 2.3% (95% CI: 1.1%-3.6%, $p=0.0002$) (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). A review of the literature relating to postmenopausal hormone use and breast cancer development was published in 1998. This includes reports on cell proliferation and endogenous hormone levels as well as epidemiological studies. The authors conclude that there is evidence of a causal relationship between postmenopausal oestrogen and progestin use and breast cancer (Colditz, 1998).

7,944 women who participated in a mammography screening programme for breast cancer in Finland were followed-up from 1987 to 1995. Information on health events and oestrogen use was obtained from biennial questionnaires and by data linking with a number of national registers. Risk of breast cancer did not increase with current use

of HRT (RR 0.57, 95% CI:0.27-1.20). Oestrogen use was classified as 'never', 'former' or 'current' and the incidence per 1000 women years was 1.8 (95% CI: 1.5-2.3), 1.6 (95% CI: 0.75-2.8) and 1.0 (95% CI: 0.47-1.9) in these groups respectively (Sourander et al., 1998). One mechanism by which it is postulated that excess endogenous and exogenous oestrogens increase the risk of breast cancer development is by increasing the proliferation of epithelial cells which have undergone partial malignant transformation (Thomas, 1984).

8.3.2 HRT and other cancers

Endometrial cancer

The endometrium is more responsive to the tumour-promoting effects of oestrogen than the breast and it is widely accepted that unopposed oestrogen therapy increases the risk of developing endometrial cancer in postmenopausal women. In the Finnish mammography study (*Section 8.3.1*) there was a significantly increased risk of endometrial cancer in current hormone users (RR 2.1, 95% CI: 1.2-3.3, $p < 0.001$) (Sourander et al., 1998).

A meta analysis of 30 published studies summarised the relative risk of endometrial cancer among HRT users receiving either unopposed oestrogen or oestrogen plus progestin. Users of unopposed oestrogen had an elevated risk of developing endometrial cancer (RR 3.3: 95% CI 2.1-2.5) and an increased risk of death (RR 2.7: 95% CI 0.9–8.0). The increased risk rises to a RR of 9.5 for 10 or more years of use and persists for several years after discontinuation of oestrogens. Data on the risks associated with oestrogen therapy combined with progestin were conflicting (Grady et al., 1995). This increased risk of endometrial cancer has always been associated with higher dose formulations of replacement oestrogens. However, a case-control studied published in 1999 involving 789 cases of endometrial cancer and 3368 population controls has reported a two-fold increase in endometrial cancer in women

using low dose oestrogen replacement, compared with women who had never used oestrogens (Weiderpass et al., 1999).

Colorectal Cancer

There is now considerable evidence for a possible role for hormonal or reproductive factors in the development of large bowel cancer in women. It has been suggested that this may be due, at least in part, to the differential effects of endogenous and exogenous oestrogens on the bile acid pool. Endogenous oestrogens are associated with lower plasma cholesterol, increased high density lipoprotein (HDL) cholesterol and increased bile acid production. Pregnancy, progestins and high dose oral contraceptives lower plasma HDL cholesterol concentrations and reduce bile acid production (Furner et al., 1989).

A case-control study was set up to investigate the conflicting role of endogenous and exogenous hormonal factors and their relationship to large bowel cancer. Female cases (n=90) and controls (n=208) were selected from an ongoing large bowel cancer study. Postmenopausal oestrogen use was found to be protective with respect to the subsequent development of large bowel cancer with an odds ratio of 0.6 (95% CI 0.33-0.99). Subsite analysis revealed the protective effect to be greatest for rectal cancer (Furner et al., 1989).

Three hundred and ninety seven (397) colon cancer deaths were observed in a cohort of 422,373 postmenopausal female participants in the Cancer Prevention Study-II initiated by the American Cancer Society in 1982. Use of exogenous oestrogen therapy as HRT was associated with a significantly decreased risk of fatal colon cancer (RR 0.71: 95%CI 0.40-0.76) and there was a significant trend ($p=0.0001$) of decreasing risk with increasing years of use (Calle et al., 1995).

A case-control study was conducted in Northern Italy between 1985 and 1992 and enrolled 709 women with incident colorectal cancer and 992 controls admitted to hospital for a wide range of acute, non-neoplastic, non-digestive tract and non-hormone related disorders. For women ever using HRT, the multivariate odds ratio was 0.40 (95% CI:0.25-0.66), with the lowest risk being associated with more than 2 years of use (Fernandez et al., 1996).

8.3.3 HRT and cardiovascular morbidity

Coronary heart disease

A review of 23 studies which was published in 1988 supports the hypothesis that reductions in cardiovascular morbidity would be expected in women receiving postmenopausal oestrogen therapy because of the effect that oestrogen has on reducing LDL cholesterol and increasing HDL cholesterol (Knopp, 1988). In the same year a study was published that showed a reduced incidence of death from myocardial infarction in oestrogen users compared with non-users (RR 0.59, $p=0.002$) and also a reduced hospitalisation rate for oestrogen users (RR 0.2, $p=0.03$) (Henderson, 1988).

The Finnish mammography study described in *Section 8.3.1* also looked at cardiovascular morbidity (Sourander et al., 1998). Current use of HRT was associated with significantly decreased cardiovascular mortality (RR 0.21, 95% CI:0.08-0.59, $p<0.001$). There was a non-significant reduction in the absolute risk of sudden cardiac death from acute MI (RR 0.45, 95% CI: 0.11-1.2, $p=0.197$).

The relation between postmenopausal hormone use and mortality in the Nurses' Health Study was reported in 1997. Included were 3,637 deaths that had occurred between 1976 and 1994 (Whitehead, 1996). The most marked reduction in deaths, adjusted for a number of confounding variables, was that due to coronary heart disease

in current hormone users (RR 0.47, 95% CI: 0.32-0.69). The reduction in mortality associated with hormone use disappeared within 5 years after stopping use and there was no increasing benefit with increasing duration of use.

This beneficial effect of postmenopausal oestrogen is less well defined where there is pre-existing coronary disease. A randomised trial was conducted to determine if oestrogen plus progestin therapy alters the risk of coronary heart disease (CHD) events in postmenopausal women with established coronary disease (Hulley et al., 1998). A total of 2,763 women were randomised to combined oestrogen progestin therapy or placebo. The primary outcome measures were the occurrence of non-fatal MI or CHD death. During an average follow-up of just over 4 years, there were no significant differences between groups in the primary outcome measures.

Thromboembolism

Evidence of an association between current use of HRT and venous thromboembolism is not clearly established. Three studies published in the same issue of the Lancet in 1996 demonstrated an increased risk of thromboembolism in postmenopausal oestrogen users. A hospital based case-control study recruited 103 cases with a diagnosis of venous thromboembolism and 178 matched controls. The adjusted odds ratio for venous thromboembolism in current users of HRT compared with non-users was 3.5 (95% CI 1.8-7.0, $p < 0.001$). No association was found with past use and risk appeared to be highest among short-term current users (Daly et al., 1996). The second study was also hospital based and looked at the association of postmenopausal oestrogen use with admission for venous thromboembolism. An analysis of 42 cases and 168 matched controls yielded a relative risk estimate of 3.6 (95% CI:1.6-7.8) for current oestrogen users compared with non-users. This estimate was strongly dose dependent (Jick et al., 1996). A prospective study documented 123

cases of PE and reported an increased risk for current oestrogen users of 2.1 (95% CI: 1.2-3.8) (Grodstein et al., 1996). Overall, the absolute risk of venous thromboembolism was low and these increases resulted in only a modest increase in morbidity.

CVA

The effect of postmenopausal HRT on the incidence of stroke is as yet unclear. The covariate adjusted difference in stroke mortality in the Finnish mammography study (*Section 8.3.1*) was of borderline significance (RR 0.16, 95% CI: 0.02-1.18, $p=0.049$). Results from a large case-control study utilising the Danish National Patient Registry showed no significant associations between unopposed oestrogen or combined oestrogen-progestagen regimens and the risk of non-fatal haemorrhagic or thromboembolic stroke in women aged 45-64 years (Pederson et al., 1997). The study adjusted for several factors which are thought to have confounded previous analyses, such as smoking, treatment for hypertension and previous OC use.

8.4 Oral contraception (OC)

8.4.1 OC use and cancer

A meta analysis 79 epidemiological studies of cancer risk associated with oral contraceptive (OC) use which had been reported in the literature between 1980 to 1994 was reported in 1995. Relative risk of cancer was estimated as a function of duration and recency of OC use. Only very small differences in cancer incidence in OC users compared with non-users were detected and the author concluded that, from a population perspective, the net effect is negligible (Schlesselman, 1995).

Breast cancer

The increased risk of breast cancer associated with OC has long been a major concern and a number of large epidemiological studies have tried to address this issue. A

report of the Nurses' Health Study in 1997 looked at the risk of breast cancer associated with OC use (Hankinson et al., 1997). There was no evidence of an increased risk of breast cancer associated with OC use in 3,383 cases of breast cancer in 1.6 million person-years of follow-up. This included data from women reporting more than 10 years of OC use (RR 1.11, 95% CI: 0.94-1.32) and five or more years of use prior to a first full-term pregnancy (RR 0.96, 95% CI: 0.65-1.43). The Royal College of General Practitioners' Study also reported no difference in the risk of breast cancer between 'ever' and 'never' users of OC ((RR 1.1, 95% CI:0.8-1.4) (Beral et al., 1999). However concern has been raised that this may provide false reassurance. The study began in 1968 when most women using the pill were married and the use of OC was relatively short-term. It does not address the issue of widespread use of the pill for many years in young unmarried women, which mimics the situation of a long gap between menarche and first pregnancy – a recognised risk factor for breast cancer (McPherson, 1999).

Ovarian cancer

Oral contraception is known to protect against ovarian cancer and this has been confirmed by the finding of a decreased relative risk of death from ovarian cancer in the Royal College of General Practitioners' Study in current and recent OC users (RR 0.2, 95% CI: 0.1-0.8, $p=0.01$) (Beral et al., 1999). A small case-control study has looked at the effect of OCs on the 10% of ovarian cancers which are hereditary. Women with mutations in either the BRCA1 or BRCA2 gene have a high lifetime risk of ovarian cancer (45% and 25% respectively). 207 women carrying a gene mutation were enrolled into the study together with 161 who were their sisters. The adjusted odds ratio for ovarian cancer associated with any past use of OC was 0.5 (95% CI: 0.3-0.8) and there was a significant trend for decreased risk with increasing duration of use (Narod et al., 1998).

Cervical cancer

A nested case-control design was used to study the association between cervical neoplasia and oral contraceptive use within a cohort of 17,000 women. After 22 years of follow-up of women in the Oxford Family Planning Association contraceptive study, the elevated risk associated with OC use appeared to be largely confined to current or recent (last use in the past two years) long-term users (OR 3.34: 95% CI 1.96-5.67). When all OC users were considered, the odds ratio was highest for invasive carcinoma (OR 4.44: 95% CI 1.04-31.6). The results suggest a possible effect of OC use on the later stages of cervical carcinogenesis, although the authors stress that residual confounding due to sexual factors or human papillomavirus infection (HPV) cannot be ruled out (Zondervan et al., 1996). For the same sexual practices, OC users are unlikely to use barrier contraception and therefore be more at risk of HPV. An increased relative risk of death from cervical cancer has also been reported in current and recent users in the Royal College of General Practitioners' Study (RR 2.5, 95% CI: 1.1-6.1, $p=0.04$) (Beral et al., 1999).

Colorectal cancer

The evidence of a reduced risk of colon cancer associated with postmenopausal exogenous oestrogen use is paralleled by a reduced risk associated with exogenous oestrogen use as oral contraceptives. The Nurses' Health Study reported on colorectal cancer in 1997. Data collected between 1980 and 1992 involving 1,012,280 person years of follow-up, indicate a reduced risk of colorectal cancer in women who had used oral contraceptives for 96 months or longer (RR 0.60: 95%CI 0.40-0.89) (Martinez et al., 1997). The case-control study conducted in northern Italy (*Section 8.3.2*) also found a reduced risk of colorectal cancer in women who had ever used OCs (multivariate odds ratio 0.58 95% CI:0.36-0.92 (Fernandez et al., 1996).

8.4.2 OC use and cardiovascular disease

Oral contraceptives were introduced into clinical practice in the 1960's and it was not long before the first case reports of thromboembolism in women taking them were received. A number of relatively small case-control and cohort studies estimated a relative risk of thromboembolism associated with OC use in the range of 2 to 11. Since then, the doses of oestrogen and progestagen in these products has been reduced and prescribing practice has changed towards the preferential use of OCs by younger women without other risk factors for cardiovascular disease. In spite of this, the results of the WHO Collaborative Study show an increased risk of venous thromboembolism associated with OC use in Europe (OR 4.15, 95% CI: 3.09-5.57). This increased risk was apparent within 4 months of starting OCs, was unaffected by duration of use and had disappeared within 3 months of stopping (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1995).

Leiden factor V mutation is a hereditary prothrombotic disorder present in 4-5% of the population and this increases the risk of thromboembolism in oral contraceptive users. A study to investigate the incidence of the relatively rare condition of cerebral sinus thrombosis in oral contraceptive users found an odds ratio of 30 in women who were OC users and carried this hereditary risk factor relative to women who had neither risk factor (de Bruijn et al., 1998).

The WHO Collaborative Study has also reported on the incidence of acute myocardial infarction in women receiving OCs. As with thromboembolism, there was early evidence of an association between OC use and myocardial infarction. Many subsequent case-control studies suggested that the association was causal. The WHO study included 368 cases and 941 matched controls. The overall odds ratio for acute myocardial infarction associated with current OC use in Europe was 5.01 (95% CI:

2.54-9.07). However, this increased risk was shown to be among women with known cardiovascular risk factors such as increased blood pressure and smoking. Very few cases were identified among women with no cardiovascular risk factors and who reported that their blood pressure had been checked before starting OC use (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1997).

The association between OC use and MI risk seen in the WHO study has not been confirmed in a UK case-control study published in 1999. The study compared 448 women who had suffered an MI with 1,728 controls and the adjusted odds ratio for current OC users was 1.40 (95% CI:0.78-2.52). There was thus no evidence of a significant association between oral contraception and myocardial infarction (Dunn et al., 1999).

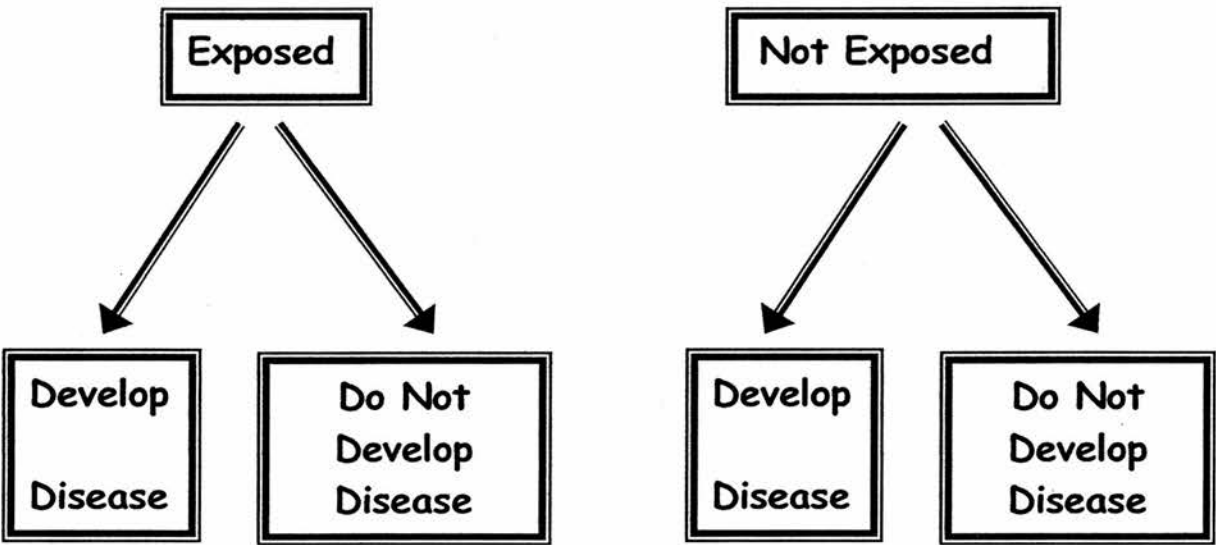
Other reports linking cardiovascular disease with OC use include a report of the Nurses' Health Study in 1997 which indicated a non-significant reduction in death from stroke (RR 0.68, 95% CI: 0.39-1.16) representing a relatively small number of cases (Grodstein et al.1997). In 1999 The Royal College of General Practitioners' Study reported an increased risk of death from cerebrovascular disease in current and recent OC users (RR1.9, 95% CI: 1.2-3.1, $p=0.009$) (Beral et al., 1999).

9.1 Types of epidemiological studies

9.1.1 Cohort studies

Cohort studies, often referred to as prospective studies, involve the selection of a group of individuals with exposure documented at the outset as exposed or non exposed, and the follow-up of both groups enables investigators to compare the incidence of a particular outcome. If a positive association exists between exposure and the outcome we would expect to see a greater incidence of the outcome in the exposed compared with the non exposed individuals. A cohort study is indicated when there is good evidence of an association between exposure and the outcome and, as follow-up is usually over a long period, loss to follow-up must be minimised. The design of a cohort study is shown schematically in *Figure 9.1*

Figure 9.1



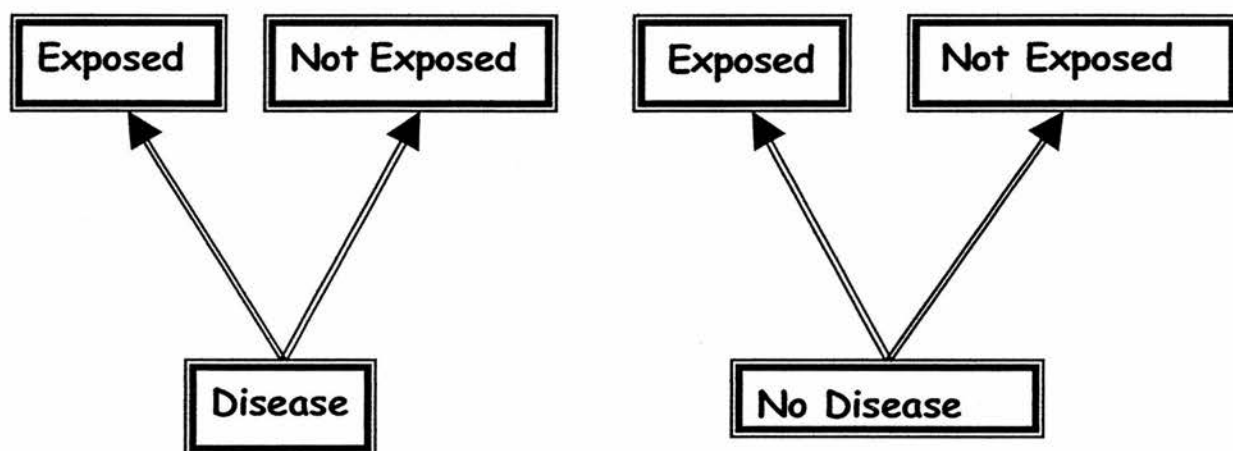
Design of a cohort study

Other types of cohort studies include, for example, dietary studies where all individuals are exposed but at different levels and studies which follow only an exposed cohort and compare it to the general population. The null hypothesis in a cohort study is that there is no association between the exposure and the outcome.

9.1.2 Case-control studies

Case-control studies, known also as retrospective studies, provide an alternative means to the cohort study of examining the possible relationship of a certain disease, or other outcome, with a particular exposure, such as a drug or an environmental factor. The key design feature of a case-control study is the selection of a group of individuals with the disease (cases) and a group of people without the disease (controls) and comparison of the two groups with respect to the exposure of interest. The proportion of cases and controls exposed can be determined by a variety of means such as interview, examination of case records or biochemical assay. The design of a case-control study is shown schematically in *Figure 9.2*.

Figure 9.2



Design of a case-control study

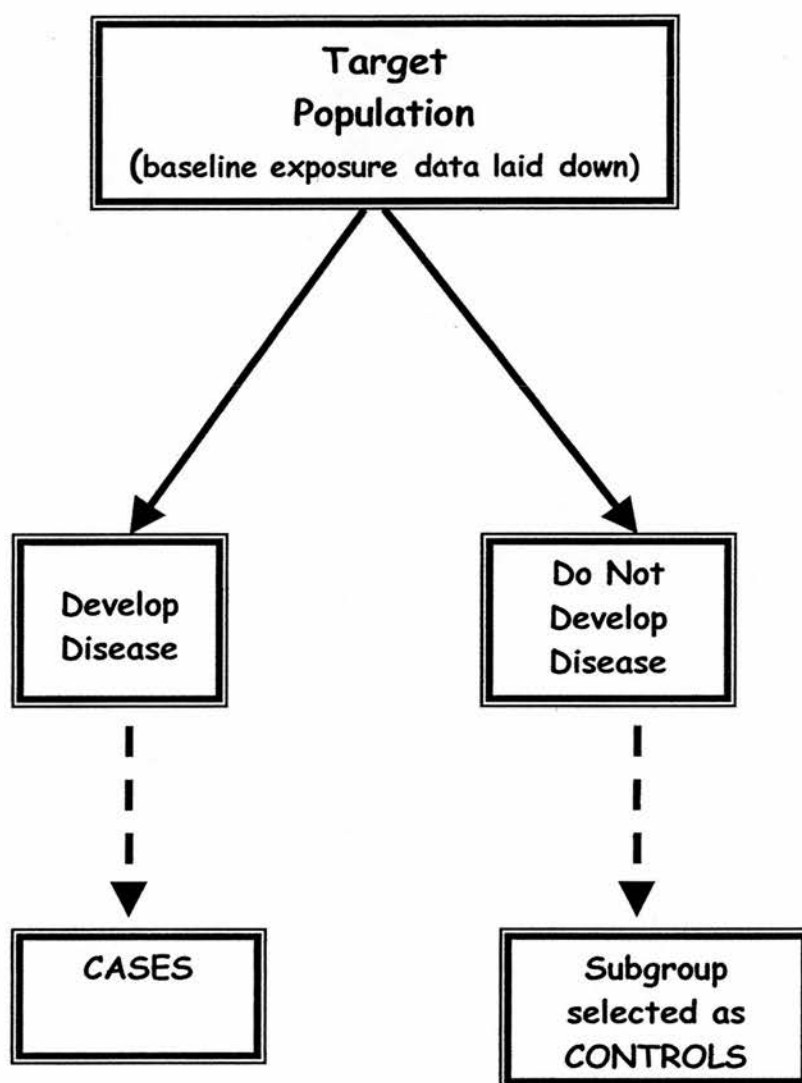
As with a cohort study, the null hypothesis in a case-control study is that there is no association between the exposure and the outcome.

The design of a case-control and that of a cohort study differ fundamentally. The former compares a group of cases and a group of controls with respect to exposure while the latter compares exposed and non-exposed individuals with respect to outcome. Because case-control studies start after the outcome event, they provide an opportunity to assess multiple causes relating to one event. They are also highly efficient in terms of the number of subjects required. However, the fact that the outcome has already occurred results in one of their disadvantages; information relating to exposure and other factors has to be collected after disease status is determined. This can lead to bias, especially interviewer bias and recall bias.

9.1.3 Nested case-control studies

The nested case-control study is a hybrid design in which a case-control study is nested within a cohort study. In this type of design a population is identified and baseline exposure data are collected, but not processed. The population is then followed over a period of time, usually years, and a percentage will experience the outcome under study and the majority will not. A case-control study is carried out in which those who experience the outcome are cases and a sample of those not experiencing the outcome are selected as controls. This is shown schematically in *Figure 9.3*.

Figure 9.3



Design of a nested case-control study

This design is free from information bias, in the same way as cohort studies are. It was developed to reduce the resource costs of cohort studies and is particularly efficient when complex analyses of biological samples are required to process the baseline data.

9.2 Methodology of case-control studies

9.2.1 Selection of cases

There are a number of basic criteria that should be applied to the selection of cases. These can be summarised as follows:

- Cases should be truly 'a case' and have had the outcome under study. Clear case definition is critical and misclassification of case status will bias the results of the study towards the null hypothesis.
- Cases should be newly incident cases. The use of prevalent cases, where the disease has been present for some time, is likely to mean that a larger number of cases are available for study. However, any risk factors identified in such a study may be related more to survival than to disease incidence. Also, the time sequence between exposure and disease will be unclear.
- Cases should be a representative sample of all arising in a defined eligible population.
- It must be possible to determine exposure and other related factors in cases.

9.2.2 Selection and number of controls

The essential characteristics of the control group follow logically from those of the cases and may be defined as:

- Controls must be truly 'non-diseased'. Definition is required of the tests to which they have been subjected.
- Controls should be representative of the population from which the cases are derived.
- Controls must be available for study so that necessary information on exposure and other factors can be determined.

The ratio of the number of cases to the number of controls is determined arbitrarily. Multiple controls of the same type are used to increase the power of the study where the number of cases is limited and cannot be increased other than by extending the recruitment period of the study. A noticeable increase in power is gained only up to a case:control ratio of about 1:4. The decision on the number of controls to use for each case is therefore based on a balance between the power requirements of the study and the availability of suitable controls.

While it is normal practice to use all available cases in a study, the number of controls available is often vastly in excess of that required. In these circumstances, a sampling procedure is undertaken to ensure that each eligible control has an equal chance of selection. Sampling should be random but may, in practice, be systematic. In random sampling, a method of random number generation is used whereby each possible control has a fixed and determinate probability of selection. One method of systematic sampling is to select controls sequentially separated by a fixed interval. Both methods require enumeration of the eligible controls.

9.2.3 Error and bias

Errors result in the value of a variable being used in the study differing from the true value of that factor. These may be non-differential, such that the inaccuracies are similar in both cases and controls with only a small possibility of there being systematic differences between the groups. Such errors include those resulting from random and biological variation and they can also be introduced during measurement, recording or computation of a variable. These result in a systematic deviation towards the null of the results.

Bias is an inaccuracy that differs in size or direction between the cases and controls. It can influence the results of a study in any direction and can produce measurements of association which are exaggerated. A number of common sources of bias can arise in a case-control study and these include:

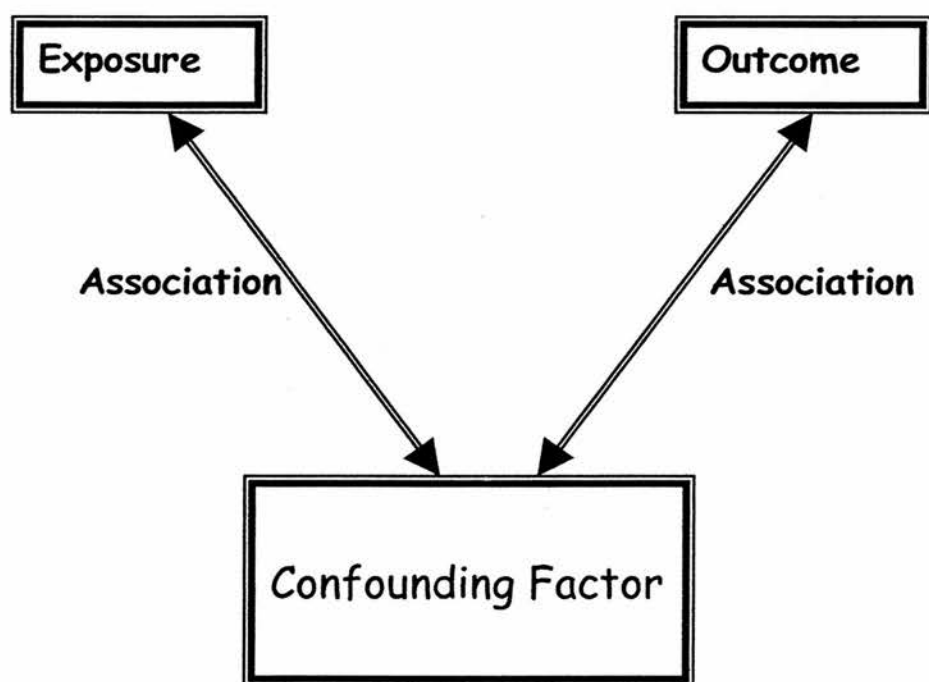
- Recall bias, arising from a subject's recall of exposure information.
- Selection bias, arising from the way in which cases and controls are selected from the target population.
- Observer bias, where the means of obtaining information on exposure and other details differs between cases and controls.

Bias also includes other areas, such as interpretation bias, publication bias and disclosure bias to name but some. In order to design a study so that bias is minimised, the choice of outcome and exposure measures must be relevant, objective, reproducible and robust. The same methods should be used under the same circumstances by the same observers for cases and controls involved in the study.

9.2.4 Confounding

One of the main problems in epidemiological studies is that of confounding. Confounding is the distortion of an exposure-outcome association brought about by the association of another factor with both outcome and exposure. These associations are independent of one another. This situation is represented schematically in *Figure 9.4*.

Figure 9.4



Schematic representation of confounding

The problem of confounding can be addressed in a number of ways, either at the level of study design or during the statistical analysis. Strategies to deal with confounding include restriction, matching, stratification and adjustment and these are discussed below.

Restriction

Restriction involves excluding those subjects, at either the design or analysis stages, who exhibit the confounding factor. For example, if smoking is a confounding factor, all patients who smoke can be excluded. This effectively leaves no possibility of confounding but reduces the generalisability of the study in that results cannot subsequently be applied to smokers.

Matching

Matching of cases and controls can be undertaken to avoid the situation where cases and controls differ in characteristics other than that being studied. This may be by group matching, where controls are selected such that the proportion with a certain characteristic is similar to the proportion in the cases, or by individual matching. In individual matching, for each case in the study, a control is selected with a similar profile of the specific variables being matched. It is important only to match on variables that are known to be risk factors for the outcome being studied and are not part of the causal pathway being investigated. Overmatching can result in difficulty in finding sufficient controls fulfilling the criteria and odds ratios artefactually close to unity. Another problem is that once controls have been matched to cases according to a particular characteristic, that characteristic cannot subsequently be studied.

An appreciable gain in precision is only realised by matching for a confounding variable that is strongly related to the exposure of interest. For less strongly related confounders, matching leads to only modest gains in precision while complicating

the study design. In the situation where the matching variable is related to exposure but not to the risk of the outcome under study, then matching reduces the efficiency of the study. In practice, age is usually a very strong confounder and case-control studies are frequently matched for age.

Stratification

As an alternative to matching, where confounding is dealt with as part of the study design, the problem can be addressed during data analysis by means of stratification. Stratification involves analysing the data in strata corresponding to different values of the confounding variable. If the association being studied is true and not due to confounding, then we would expect to see this association in each of the strata in the analysis. Problems can arise as a result of small numbers in strata and the consequent instability of estimates. In these circumstances it is necessary to produce a summary measure of association, in the form of a weighted average of the stratum-specific estimates.

Adjustment

An alternative method for dealing with confounding variables is to analyse the data by means of a mathematical model that takes the outcome under consideration as the dependent variable and includes both the postulated causal factor and confounding factors in the equation. The covariates may be continuous, dichotomous or categorical and the logistic regression model can be applied, or conditional logistic regression if a matched design is being used. This allows the effect of the exposure of interest on the incidence of the studied endpoints to be determined having adjusted for confounding variables.

9.3 Analysis of observational studies

9.3.1 Estimating risk

The incidence of a disease in a population is known as the **absolute risk**. Absolute risk is an indicator of the magnitude of the risk in a group of exposed people, but it does not take into account the risk of disease in non exposed individuals. It therefore cannot indicate whether or not exposure is related to an increased risk of the disease. To determine whether such an association exists between exposure and the disease, we consider the ratio of the risk of disease in exposed individuals over the risk of disease in non-exposed individuals. This ratio is the **relative risk**.

$$\text{Relative risk} = \text{Risk in exposed} / \text{Risk in non-exposed}$$

The relative risk can be calculated directly in cohort studies but in case-control studies the incidence of disease in the exposed and non-exposed population is not known and the relative risk cannot be calculated directly. An alternative measure, the **odds ratio**, is therefore calculated in case-control studies.

In general terms, the odds of an event can be defined as the ratio of the number of ways that the event can occur to the number of ways the event cannot occur. If the probability of an event occurring is P , then the odds that the event will occur are defined as $P/(1-P)$. In a case-control study, the odds ratio is defined as the ratio of the odds that the cases were exposed to the odds that the controls were exposed. If the exposure is not related to the disease, the odds ratio will be 1. If the exposure is positively related to the disease, the odds ratio will be greater than 1. If the exposure is negatively related to the disease, the odds ratio will be less than 1.

Calculation of relative risk and odds ratio is shown schematically in *Figure 9.5* for a cohort study.

Figure 9.5

	Exposed	Not exposed
Cases	a	b
Non cases	c	d

$$\text{Relative Risk} = \frac{\text{Risk in exposed}}{\text{Risk in non exposed}}$$

$$= \frac{a/(a + c)}{b/(b + d)}$$

$$\begin{aligned} \text{Odds Ratio} &= \frac{\text{Odds that exposed person develops disease}}{\text{Odds that a non exposed person develops the disease}} \\ &= \frac{a/c}{b/d} = \frac{ad}{bc} \end{aligned}$$

Calculation of the Relative Risk and Odds Ratio

When the disease outcome is rare, the odds ratio is a good approximation of the relative risk in the population. Cases and controls must be representative, with regard to exposure, of people with and without the disease respectively in the population from which they were drawn and sampling fractions must not depend on exposure. The relationship between odds ratio and relative risk for different disease frequencies is illustrated in the following calculations for two hypothetical cohort studies (*Figures 9.6 and 9.7*).

Figure 9.6

	Exposed	Not exposed
Cases	50	25
Non cases	50	75

$$\text{Relative Risk} = (50/100)/(25/100)$$
$$= 2$$
$$\text{Odds Ratio} = (50 \times 75)/(25 \times 50)$$
$$= 3$$

Comparison of OR and RR in a cohort study where disease is frequent

Figure 9.7

	Exposed	Not exposed
Cases	200	100
Non cases	9800	9900

$$\begin{aligned}\text{Relative Risk} &= (200/10000)/(100/10000) \\ &= 2\end{aligned}$$

$$\begin{aligned}\text{Odds Ratio} &= (200 \times 9900)/(100 \times 9800) \\ &= 2.02\end{aligned}$$

Comparison of OR and RR in a cohort study where disease is rare

9.4 Design of current study

9.4.1 Introduction

The current study was designed as an observational study comprising a number of case-control studies, nested in cohorts formed from the target population. Baseline data with respect to the exposures of interest (exogenous oestrogens) were obtained routinely for the hospital case-notes from the patient at presentation. A strong feature of the study design is that cases and controls are derived from the same target population. This comprised all women who had presented to the Edinburgh Breast Unit between 1989 and 1994 inclusive, and for whom there is a computerised data record. This record was extracted from the database and included in the dataset which was linked with the SMR6 cancer registration and GRO death records. Output from the linkage procedure consisted of two datasets; one dataset consisted of records from the EBU database which had not been linked with a SMR6 or GRO record and the other consisted of EBU records and one or more SMR6 records and/or a GRO record with which they had been linked.

The exposures of interest were exposure to exogenous oestrogens, as oral contraception (OC) or hormone replacement therapy (HRT), both of which were classified at the time of presentation as current, previous or never. Information on duration of exposure was available for some, but not all of cases. A single exposure category (oestrogen exposure/no oestrogen exposure) was used in the analyses.

9.4.2 Selection of cases and controls

Cases were drawn from the set of linked records and were those subjects:

- i) who had died and the registered cause cited on the death certificate, as either the underlying cause or a secondary cause, was ischaemic heart disease (IHD), cerebrovascular disease or thromboembolic disease according to the ICD9 classifications in *Table 9.1*.

Table 9.1

Cause of Death	ICD9 Classification
Ischaemic Heart Disease	410, 411, 413, 414
Cerebrovascular Disease	430-437
Thromboembolism (PE/DVT)	451, 4151

Note: where an ICD9 code is given to 3 digits, all further subclassifications are included e.g. 451 includes 4510-4519 inclusive

Causes of death endpoints for study

- ii) who were registered on SMR6 with a new primary cancer, since entry to the study, at a site identified as being potentially relevant to oestrogen exposure, as defined in *Table 9.2*

Table 9.2

Malignancy	ICD9 Classification
Colorectal cancer	153, 154
Liver cancer	155
Hodgkin's disease	201
Endometrial cancer	182
Ovarian cancer	183

Sites of new primary malignancy as endpoints

Breast cancer was not included as an outcome for the current study as it was considered that inclusion of the incidence of breast cancer in a population presenting to a Breast Unit would require careful consideration of, and adjustment for, other variables known to be risk factors for the development of breast cancer. These include age at menarche, parity and family history. Hodgkin's disease has been included as an endpoint because there are epidemiological data which suggest an involvement of reproductive, and thus hormonal factors, in its pathogenesis in women (Bourdais-Mannone et al., 1999).

Whether or not a case had a diagnosis of breast cancer at presentation was determined by referring back to the dataset of linked records and cross-checking the date of presentation with the date of diagnosis of breast cancer, if this was present. This enabled distinction between subjects who presented with a diagnosis of breast cancer and those who developed breast cancer at a later date.

Controls were a sample of women, individually matched with cases for year of birth and the presence or absence of a diagnosis of breast cancer at presentation, who had none of the above endpoints. Separate groups of controls were selected for each of the endpoints. The populations from which the controls were drawn are illustrated in *Figure 9.8*.

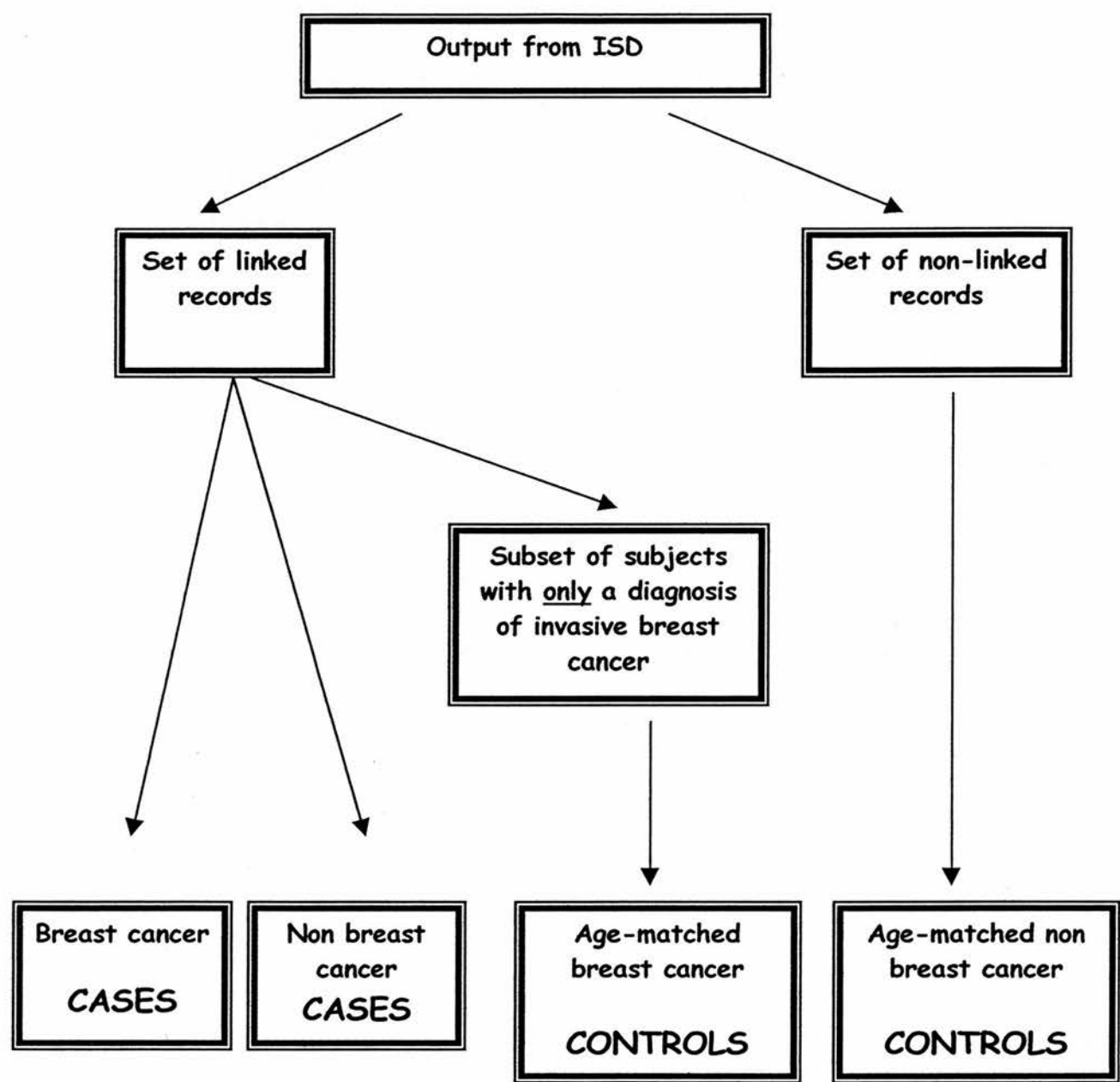
Controls without a diagnosis of breast cancer at presentation were drawn from the set of EBU records that had not been linked with an SMR6 or a RGO record. Thus, the non breast cancer control set was already available as a result of the linkage process. Controls with a diagnosis of breast cancer at diagnosis were drawn from the set of linked records. To be eligible as a breast cancer control, a subject was required to be alive with a diagnosis of invasive breast cancer within one month of a single presentation to EBU between 1989 and 1994. No other malignancy or hospital admission should be present. This process could only be carried out by manual checking of the entire set of linked records, marking those that satisfied the criteria and copying the marked set to a new table. This was the set of breast cancer controls. The irregular structure of the records made it impracticable to use programming methods to select the control set.

A similar process was implemented to randomly select the controls from each of the two control datasets. Consider the situation where 5 breast cancer cases have the year of birth 1945. For a case to control ratio of 1:2 it is necessary to randomly select 10 controls from the set of breast cancer controls with the year of birth 1945. The following procedure was adopted:

- A query was run in Microsoft Access to select all records in the breast cancer control set with the year of birth 1945.
- If this process yielded, say, 40 possible controls (numbered sequentially from 1 to 40) the random number generator in Microsoft Excel was implemented to randomly select 10 numbers between 1 and 40.
- These numbers identified the 10 controls required.

If matching by year of birth failed to yield sufficient possible controls, additional controls were selected from the next following year. The selected case:control ratio of 1:2 was that considered to be the optimum value in terms of power of the study and practicality of obtaining the relevant information from the EBU database within the required timeframe.

Figure 9.8



Schematic representation of populations from which cases and controls were selected

9.4.3 Discussion of method of selection of controls

Age has a strong association with the outcomes and exposures and is therefore, by definition, a confounding factor. Frequency matching of cases and controls within 5 year age bands is one option for taking this confounding effect into account. However, for logistic reasons related to the structure and subsequent manipulation of the linked data as supplied by ISD, the decision was taken to match cases and controls individually on year of birth. Date of presentation was not available on the listings of cases (Section 9.5.4) and therefore age was not used as a matching variable. For the same reason, cases and controls were not matched on duration of follow-up and therefore controls were not defined to be free of disease when the case was diagnosed.

Cases and controls were also matched on the presence or absence of breast cancer. This was important because of the effect the presence of breast cancer may have on the endpoints being studied and was implemented by matching the controls to the cases on breast cancer diagnosis as described. Had all the controls been selected from the set of unlinked records then, by definition, none of the controls would have had a diagnosis of breast cancer at presentation. The diagnosis of breast cancer was restricted to invasive breast cancer.

Exclusions

Cases and controls identified in the target population were excluded from the eligible population for analysis for any of the following reasons:

- i) The subject did not have a record on the EBU database. This was the case when a mismatch had occurred between the EBU database and the ISD combined database.

- ii) A record was present on the database but no presentation details were recorded.
- iii) For a new primary cancer endpoint, if a previous malignancy at any site was noted prior to presentation at EBU this case was excluded. Previous malignancies did not exclude cases from the analysis of the other cause of death endpoints, as this would have required checking every case for an entry on the cancer registration database (SMR6).

9.4.4 Sources of bias

Selection bias was minimised within the study by selecting all available cases with the outcomes of interest ascertained from the data linkage with cancer registration and death registration databases. Controls, individually matched with cases by year of birth and presence or absence of breast cancer diagnosis, were randomly selected from the relevant control sets. Information bias was excluded by the prospective, nested case-control design.

9.5 Data linkage

9.5.1 Preparation of data for linkage

Ascertainment of cases in the study was by means of record linkage of the target population with the cancer registration database (SMR6) and the GRO death registration records held on the combined database at ISD. The background and a detailed discussion of the record linkage procedure are given in *Chapter 3*. The linkage with the EBU data was carried out by ISD in February/March 1999, at which time death registration on the combined database was complete to March 1998 and cancer registration on SMR6 was complete to the end of 1996.

The data for linking with the combined database were extracted from the EBU database for patients presenting in the years 1989 to 1994 inclusive. The records were extracted into a Microsoft Excel file by a member of staff at EBU (PD). The variables which were extracted for each presentation are shown in *Table 9.3*. Postcode is formatted into 2 variables in the EBU database and these were combined into a single field for exporting.

Table 9.3

Variable
surname
first name
date of birth
postcode of residence
date of presentation

Variables extracted from EBU database for linking with SMR6 and GRO records

The total number of data records included in the linkage was 27,089, corresponding to the number of presentations at EBU between 1989 and 1994 inclusive. It is not uncommon for subjects to present for the unit on more than one occasion. Before the linkage with SMR6 and GRO records in the combined database was undertaken, staff at ISD performed an internal linkage to remove instances of duplicate presentations.

A total of 19,410 individual patients were then available for linking. Some manipulation of the data was required prior to linkage to correct inconsistencies in the formatting of postcode and dates of birth in the spreadsheet. This was also carried out by staff at ISD.

Linkage of the data to hospital inpatient statistics (SMR1) was not carried out in this part of the study because only mortality data and new primary cancers were of interest.

9.5.2 Matching score

During the linkage procedure at ISD, the data were reviewed to determine the level of weighted probability which gave as near 100 to 1 absolute odds that the matched records belonged to the same individual. Inspection revealed that weighted probabilities above 27 indicated true matches, those under 23 were mismatches and those between 23 and 27 represented a combination of true and mismatches. All scores of 23 and above were included in the linkage output. All records selected as cases or controls had their data records accessed on the EBU database and the validity of the match was thus checked at this stage.

9.5.3 Output of linked data

The linked data were compressed by ISD into an ASCII file onto a 3.5" disk. On receipt by the author the file was expanded and exported into a Microsoft Access database. The ISD output included two files; unlinked EBU records and linked EBU records with the corresponding SMR6 and/or GRO records. This second file of linked records comprised information relating to 4,122 patient records. All fields in the imported file were text fields. The structure of the records differed depending on the source of the record (EBU, SMR6, GRO). This is illustrated in *Table 9.4*.

Table 9.4

Field No.	Description	Size	Format/Source
1	Linkno	8	Unique identifier allocated to all records by linkage procedure
2	Record Type	2	00=EBU, 06 = SMR6, 09=GRO
3	Presentation Date	6	EBU record
	Registration Date	6	SMR6 record
	Date of Death	6	RGO record
4	Surname	12	Text
5	Forename	12	Text
6	Maiden Name		SMR6 and RGO records
7	Date of birth	6	DDMMYY
8	Sex	1	2 = Female
9	Postcode	7	
10	Case Reference No.	10	
11	Linkage weight	4	EBU record
	Cancer site code	4	SMR6 record
	Primary cause of death	4	RGO record
12	Secondary causes	14	RGO record

Structure of linked dataset showing records from 3 different sources – EBU, SMR6, GRO

9.5.4 Ascertainment of cases and selection of controls

Queries were run on the Microsoft Access database of linked records to identify the endpoints defined in *Table 9.1*. Listings were obtained for each of the cause of death endpoints being studied and for all new, non-breast primary cancers. These data listings comprised the population from which the cases in the study were drawn. Queries in Microsoft Access were run on both the linked and not linked datasets to select the controls for the study, as described in *Section 9.2.2* above.

9.5.5 Data collection

Data collection was carried out by the author at the Western General Hospital where access to the EBU database was available. Cases and controls were identified on the EBU database by name and date of birth, the relevant information obtained from the presentation screen and entered directly into a Microsoft Access database which had been previously created on a laptop computer. A description of this database is given in *Table 9.5*. Age at presentation was calculated from the date of presentation and date of birth.

Table 9.5

Field Name	Description
Linkno	Unique linkage no assigned by ISD
d_pres	Date of presentation to EBU
Menstrual	1=premenopausal 2=postmenopausal
dob	Date of birth
hx	Hysterectomy: 1=YES 2=NO
ox	Oophorectomy: 1=BILATERAL 2=UNILATERAL 3=NO
oc	Exposure to OC: 1=CURRENT 2=PREVIOUS 3=NEVER
oc_dur	Duration of exposure to OC
hrt	Exposure to HRT: 1=CURRENT 2=PREVIOUS 3=NEVER
hrt_dur	Duration of exposure to HRT
Diagnosis	Diagnosis: 1=BREAST CANCER 2=BENIGN
Oestrogen	Oestrogen exposure: 1=EXPOSED 2=NOT EXPOSED 9=NOT KNOWN

Structure of database for entering exposure data obtained from EBU database

During data collection it was noted that the recording of the presence or absence of hysterectomy (Hx) and oophorectomy (Ox) on the EBU database was inconsistent. The following consistency rules were implemented at data entry:

- If Hx = NO and Ox is missing, then Ox = NO
- If Hx=YES (1 or H) and Ox is MISSING, then Ox = NOT KNOWN
- If Hx is MISSING and Ox is MISSING, then Hx is NOT KNOWN and Ox is NOT KNOWN

Menstrual status is recorded on the EBU database as REGULAR, IRREGULAR, STOPPED. For the purposes of this study, REGULAR AND IRREGULAR were both coded as PREMENOPAUSAL.

9.5.6 Data checking procedures

Linkage validation

No systematic validation of the linkage process was undertaken. However, the process of accessing the record on the EBU database to obtain the necessary information relating to oestrogen exposure was, in effect, a method of validation. The search was by name as recorded on the ISD combined database and the appropriate record would not be found on the database unless this was identical to the name as it was recorded on the EBU database. Date of birth was always verified to ensure that the correct record had been accessed. If a record could not be found on the EBU database, possible alternative spellings were tried, to ensure that a true match was not being missed – e.g. Miller for Millar, Johnston for Johnstone, Ann for Anne,

McDonald for Macdonald. If a record was not identified on the database at the first attempt a further attempt was made at a later date as an additional check that it was not present on the EBU database. If, after following these procedures, a record was not identified on the EBU database, it is reasonable to assume that this was an ISD record that had been mismatched with an EBU record.

Consistency checks

When data collection was complete, a number of queries were run on the Microsoft Access database to identify records with inconsistent or implausible data. Such inconsistencies may arise from data entry errors or from errors on the database. Data for records identified by this procedure were re-checked with the EBU database. The consistency checks performed were as follows:

- Age < 21 or > 100 years
- Age > 60 years and premenopausal
- Premenopausal but current or previous HRT
- Postmenopausal but current oral contraception
- Duration of HRT or OC recorded but status = NEVER

The results of the data checking procedures are described in *Chapter 10*.

9.6 Statistical methods

Various calculated fields were created before the data were subjected to statistical analysis. These are described in *Table 9.6*. Matching of cases and controls had been implemented by year of birth (or the following year) and breast cancer status at presentation and a single matching variable for the analysis was created by combining the 5 year age-band stratification and the presence or absence of breast cancer at presentation. This variable took the value 1-22. The data were exported as

separate text files for each of the outcomes under study and were thus presented in the appropriate matched sets (by 5 year age band and breast cancer status) for each analysis.

Table 9.6

Calculated Field Name	Description
Age	Age calculated from (d_pres – dob)
Age Strata	Age stratified into 5-year age bands 1=<40,2=40-44,3=45-49,4=50-54,5=55-59,6=60-64, 7=65-69,8=70-74,9=75-79,10=80-84,11=>84
Oestrogen	Oestrogen exposure: 1=CURRENT, PREVIOUS 2=NEVER
Outcome_ihd	0 = control for IHD 1 = case for IHD
Outcome_t	0 = control for PE/DVT 1 = case for PE/DVT

Description of calculated fields for the case-control analyses

Statistical analysis was by standard methods of analysis for matched case-control studies using conditional logistic regression, which enables the investigation of the relationship between one or more predictors and a binary outcome/response. For each outcome studied, a point estimate of the odds ratio (i.e. relative odds of that outcome for exposed versus non exposed individuals) was determined, together with a confidence interval around that estimate The logit model assumes a binomial error

distribution and allows information about the relationship between outcome and exposure to be obtained.

$$\text{Odds} = p/(1-p)$$

$$\text{Logit} = \log \text{ odds} = \log[p/(1-p)]$$

Where p = proportional response

$$\begin{aligned}\text{Logit 1} - \text{Logit 2} &= \log[p/(1-p)] - \log[p_2/(1-p_2)] \\ &= \log [(p_1/(1-p_1)) / (p_2/(1-p_2))] \\ &= \text{Odds Ratio}\end{aligned}$$

The logistic regression equation takes the form:

$$\text{Log}[p/(1-p)] = a + b_1x_1 + b_2x_2 + b_3x_3 \dots\dots$$

Where the x variables represent exposure factors and the b terms are the coefficients

The conditional logistic regression model is used to analyse data where controls have been matched to cases. In this model the results are analysed in terms of case-control sets rather as individual subjects. The method avoids having to estimate a large number of fixed effect parameters corresponding to the baseline responses for the sets induced by the matching. Statistical analysis was implemented in the Conditional Logistic Regression module of the Egret® (Serc) statistical software.

9.7 Changes to study design

The study was originally planned such that, after the identification of cases and controls, hospital case-notes would be reviewed to obtain the information recorded at presentation which was to be included as covariates in the analysis. The variables which it was planned to study were:

- * Menstrual status
- * Occurrence of hysterectomy/oophorectomy
- Smoking history
- Previous relevant medical history
- * Oestrogen exposure as OC / HRT
- * Duration of oestrogen exposure
- Occurrence of previous cancer at any site

In the event, it transpired that only a very small percentage of case-notes were available and these related exclusively to cancer patients. This was largely due to the fact that hospital policy is now to destroy case-notes of discharged patients with benign disease after a period of 3 years has elapsed since death.

Therefore the only available source of the information required was the EBU database. This was known to be incomplete for parameters other than menstrual status (periods regular, irregular or stopped) and use of OC and HRT (current, previous, never) for the years of interest in the study (1989 – 1994). The data collected for the study had to be restricted to the variables in the above list marked with an asterisk (*). Information on duration of OC/HRT use and the presence of hysterectomy and oophorectomy was scanty but was nevertheless collected where available.

10.1 Results of linkage of EBU data with SMR6 and GRO records

After inspection of the linked data file by the operator from ISD, it was determined that a probability weight of over 27 corresponded to a 100/1 absolute odds of a true match, a probability weight of less than 23 corresponded to a mismatched record and probability weights of between 23 and 27 were a combination of true matches and mismatched records. All records linked with a probability weight of 23 or over were output to the linked dataset. No specific action was taken at this stage to validate whether or not matches were correct. Using these criteria, the linkage procedure resulted a total of in 9,137 data records comprising EBU records for 4,022 women and the SMR6 cancer registration and GRO death records with which they had been matched.

10.2 Number of events

The number of deaths identified from linkage with GRO records and corresponding to the causes of death considered for inclusion in the study are shown in *Table 10.1*. The number of all new primary malignancies identified from linkage with SMR6 are presented, classified by site of cancer, in *Table 10.2*. Patients developing new primary cancers at more than one site during the follow-up period will appear in more than one site classification. Breast cancer has been excluded as an endpoint in the current study.

Table 10.1

Event	ICD9 Classification	No. of events
Ischaemic Heart Disease	410, 411, 413, 414	153
Cerebrovascular Disease	430-439	141
Thromboembolism (PE/DVT)	451, 4151	43

Number of events identified from linkage of EBU data with GRO records

Table 10.2

Site of primary malignancy	ICD9	No. of Events
Lip and oral cavity	140 - 149	3
Digestive organs	150 - 159	103
Respiratory and intrathoracic	160 - 165	64
Bone, connective tissue and skin	170 - 173	158
Ovarian	184	14
Endometrium	182	36
Other and unspecified sites	190 -195	19
Lymphatic and haematopoietic tissue	200-208	33
TOTAL		430

Number of new primary malignancies at all sites (excluding breast) identified from linkage of EBU data with SMR6 records

The broad classification of sites of new primary malignancy noted in *Table 10.2* are further broken down in *Table 10.3* into those sites which are thought to be most influenced by endogenous and exogenous oestrogen exposure and which are therefore of most interest for the purposes of the current study.

Table 10.3

Cause of Death	ICD9 Classification	No. of events
Colorectal cancer	153, 154	79
Liver cancer	155	2
Hodgkin's disease	201	0
Endometrial cancer	182	36
Ovarian cancer	183	65

Number of new primary malignancies at sites of interest for the current study.

10.3 Scope of the current study

Unforeseen delays in the preparation of the data for linkage and the processing of the linkage procedure itself resulted in time constraints necessarily being placed on the analyses to be undertaken. Access to the EBU database was also difficult with computer terminals rarely freed up from routine use. The scope of the study was therefore confined to those outcomes considered to be the most relevant. Decisions

on the outcomes to be analysed were made before data collection was started. With regard to cause of death, ischaemic heart disease and thromboembolism were selected for study. The number of cases of liver cancer and Hodgkin’s disease was negligible and the analysis of new primary malignancies was restricted to endometrial cancer.

10.4 Data validation

Table 10.4 presents the results of the consistency checks that were run on the data collected from the EBU database. These results indicate that there were 14 records where the recording of menstrual status can be considered unreliable.

Table 10.4

Detail	Number of Records
Age < 21 or > 100 years	0
AGE > 60years but PREMENOPAUSAL	7
PREMENOPAUSAL but HRT = PREVIOUS or CURRENT	7
POSTMENOPAUSAL but OC = CURRENT	0
Duration of HRT given but HRT = NEVER	0
Duration of OC given but OC = NEVER	0

Data inconsistencies identified in EBU database

10.5 Overview of results of data collection

The following sections consider only the results for the mortality endpoints of ischaemic heart disease and thromboembolism. Results with respect to the incidence of endometrial cancer are presented in *Section 10.9*.

The linkage procedure and selection of controls (*Chapter 9, Section 9.4.2*) identified a total of 196 cases and 320 controls for the endpoints of ischaemic heart disease and thromboembolism. However, as data collection progressed, it became clear that not all of the cases and controls selected had data recorded on the EBU database. Two main reasons for this were identified:

- i) A subject identified by the linkage as experiencing an event did not have a record on the EBU database. This was indicative of an incorrect matching between the EBU database and the combined SMR6/GRO database and serves as an internal validation of the matching procedure.
- i) A subject was registered on the EBU database and the record was identified but no presentation data were present and therefore no information on oestrogen exposure was available. The reason for these data not being recorded was not apparent.

A total of 21 records could not be found on the EBU database, indicating that they were not valid matches between the ISD combined database and the EBU database. A further 16 records identified on the EBU database had no presentation details associated with them. Thus, a total of 170 cases and 309 controls were available for data collection and inclusion in the statistical analyses. As a result of the procedure for selecting controls, 31 patients had been selected as controls for both endpoints. The effective number of controls available for study was therefore 340 giving an overall case:control ratio of 2, although of course each analysis proceeded

independently. The number of cases for inclusion in the analyses of the two cause of death endpoints is presented in *Table 10.5*.

Table 10.5

Cause of Death	No. of Cases
Ischaemic Heart Disease	130
Thromboembolism (PE/DVT)	40

Number of cases available for analysis on the EBU database

Table 10.6 presents an overview of the data that were available for the 479 cases and controls and also indicates where data are missing. The percentages in the final column refer to the percentage of the total for which data are available.

Table 10.6

Variable	Number		%	
	Cases (170)	Controls (309)	Cases	Controls
Menstrual Status:				
Premenopausal	16	26	9.4	8.6
Postmenopausal	154	274	90.6	91.3
Missing	0	9		
OC:				
Current	1	0	0.7	0
Previous	23	49	15.8	17.6
Never	122	230	83.6	82.4
Missing	24	30		
Median duration of OC	3.5 years			
(range)	(1 month – 20 years)			
Missing	8	1		
HRT:				
Current	8	12	6.3	4.7
Previous	1	3	0.8	1.0
Never	118	243	93.0	94.2
Missing	43	51		
Median duration of HRT	2 years			
(range)	(2months – 5.5 years)			
Missing	2	0		
Exposure to OC or HRT:				
Yes	29	52	23	20.3
No	97	204	77	79.7
Missing	44	53		

Overview of data collected from EBU database

% refers to percentage of available data, excluding missing values

10.6 Power of the study

10.6.1 Definition of power

The power of a study is the probability that the study can demonstrate a significant association, given that an association exists. Power is defined as $1-\beta$, where β is the probability of not detecting a significant difference where a true difference exists. β is thus the risk of a false negative result and is known as the Type II error. Several factors affect the power of a study:

- The strength of the true association – the larger the true difference between groups the easier it will be to detect.
- The frequency of outcome, as given by the number of cases and controls.
- The prevalence of exposure among cases and controls. The maximum power is achieved when about one half of all the subjects are exposed.

In calculating the sample size for a study, the power is often set at 0.8. This means that the study is designed so that the chance of detecting a significant difference, if the true difference exists, is 80% and we accept that we will miss the true difference 20% of the time and get a false negative result. Another factor of relevance when considering sample size is the significance level, α . This is the cut-off point used to determine whether the association found is statistically significant and is most frequently set at the $p=0.05$ level. α is called the Type I error and is the probability of detecting a 'significant difference' when no true difference exists – i.e. α represents the risk of detecting a false positive result.

10.6.2 Calculation of power in the current study

Estimations of the ability of the current study to detect an association between exposure and risk of the outcomes under study were derived for each of the endpoints from the number of cases available for analysis. *Table 10.6* indicates that 52 of 256 controls (20.3%), for whom the information was available, were exposed to oestrogen as either OC or HRT. This exposure level has been used in the power calculations. The calculation has been carried out for case-control studies in which there are two matched controls for every case and for a range of values of the correlation coefficient of exposure between cases and controls (0.25, 0.5 and 0.75). The calculation was performed using Arcus Quickstat Biomedical (Research Solutions), and the figures obtained represent the minimum number of cases required to have an 80% chance of detecting a true effect with a Type I or alpha error (risk of false positive result) of 0.05, for a range of OR between 2 and 10.

Table 10.7

Odds Ratio	Correlation Coefficient for Exposure between Cases and Controls		
	0.25	0.5	0.75
	Minimum Number of Cases		
2	160	240	480
3	61	96	203
4	38	61	135
5	28	46	106
6	23	38	90
7	19	33	80
8	17	30	73
9	15	27	68
10	14	25	64

Minimum sample size required in terms of detectable odds ratio for exposed compared with non exposed individuals for different correlation coefficients of exposure between cases and controls

Thus, from *Table 10.7* it can be seen that for the endpoint of ischaemic heart disease (130 cases), under the conditions described, the study has an 80% power to detect an odds ratio of 2 to 4 between exposed and non exposed individuals. For the outcome of thromboembolism (40 cases), the study is sufficiently powered to detect an odds ratio of 4 and 6 respectively for exposure correlation coefficients of 0.25 and 0.5 respectively. However, *Table 10.7* also shows that for an exposure correlation coefficient of 0.75, the number of thromboembolic events is insufficient to detect an odds ratio of exposed to non exposed individuals of 10.

10.7 Results for ischaemic heart disease

Table 10.8 presents the results for ischaemic heart disease in a simple 2x3 table of numbers of cases and controls and their exposure status to exogenous oestrogen, as either HRT or OC. Note that 38 cases and 48 controls were excluded from the statistical analysis because the EBU presentation data did not include the necessary information on oestrogen exposure.

Table 10.8

	Cases	Controls	Total
Exposed	23	37	60
Not exposed	69	177	246
Not Known	38	48	86
Total	130	262	392

Numbers of exposed, non exposed and exposure unknown cases and controls for the endpoint of ischaemic heart disease

Ninety two cases of ischaemic heart disease with known oestrogen exposure status were included in the final analysis together with 214 controls. Cases and controls were matched in the analysis by 5 year age band and breast cancer status at diagnosis. The data as presented for statistical analysis are attached as *Appendix 6*. Statistical analysis was by the conditional logistic regression module of the statistical package Egret® (Serc). Menstrual status was not used as a covariate because the consistency checks carried out on the database had indicated that the recording of this variable on the EBU database was unreliable. The covariate under investigation was that relating to oestrogen exposure. It is worth remembering that when the conditional logistic regression model is used to estimate the odds ratio, the only matched sets that participate in the estimation of the odds ratio for a covariate, V, are those in which two subjects differ in their values for V.

The analysis of the influence of exogenous oestrogen exposure on death from ischaemic heart disease yielded an odds ratio of 1.001 (95% CI: 0.991-1.012, $p=0.822$) for exposed compared with non exposed individuals. Thus the results show no indication of an association between oestrogen exposure and death from IHD in this study.

10.8 Results for thromboembolism

Table 10.9 presents the results of the oestrogen exposure status for the cases and controls for the endpoint of thromboembolic death, defined as PE or DVT.

Table 10.9

	Cases	Controls	TOTAL
Exposed	6	15	21
Not exposed	28	51	79
Not Known	6	12	18
TOTAL	40	78	118

Numbers of exposed, non exposed and exposure unknown cases and controls for the endpoint of thromboembolism

Six cases and 12 controls were excluded from the statistical analysis because oestrogen exposure data were not available. Thus of 40 cases and 78 controls identified, 34 cases and 66 controls were available for the analysis. As before, a matching variable was created from the age stratification and the breast cancer status at presentation. The data for analysis are attached as *Appendix 7*.

Statistical analysis by conditional logistic regression yielded an odds ratio of 0.411 (95%CI: 0.097-5.223, p=0.737) for exposed compared with non exposed individuals and does not indicate an effect of oestrogen exposure on death from thromboembolism, as PE or DVT, in this study.

10.9 Results for endometrial cancer

A total of 34 of the 36 cases of endometrial cancer which were identified from the data linkage had a data record on the EBU database. However, it became apparent during the collection of exposure data that a significant number of these cases related

to endometrial cancers which had a date of onset preceding the date of presentation to the EBU. The linked dataset contained all primary cancer registrations for an individual and was not limited to those occurring from 1989 onwards. Those cases of endometrial cancer with a date of onset preceding the date of presentation to the EBU were not eligible for inclusion in the analysis. Thus a total of 17 cases were excluded, leaving only 17 available for analysis. This number was insufficient to justify proceeding with statistical analysis at this time.

11.1 Study power: comparison with published studies

The calculations of study power which are presented in *Chapter 10, Section 10.6.2* suggest that the current study is sufficiently powered to detect an odds ratio of between 2 and 4 at the 5% level of significance, between individuals exposed and not exposed to oestrogen as HRT or OC, for a maximum correlation coefficient of oestrogen exposure in cases and controls of 0.75. Published studies of the relative risk of myocardial infarction in users of HRT indicate that the greatest benefit is seen in current users who have pre-existing cardiovascular risk factors (OR 0.51, 95% CI:0.45-0.57) (Grodstein et al., 1997). A striking decrease in cardiovascular mortality was observed in a population study of 7,944 women (adjusted risk ratio 0.21, 95% CI:0.08-0.59) (Sourander et al., 1998). One important difference between the current study and many of the published studies is that data are not available on smoking history and cardiovascular risk factors and these are therefore unmeasured confounders.

The sample size calculations for the current study (*Table 10.7*) indicate that the number of cases of death from ischaemic heart disease identified in this study should be sufficient to detect differences of the order of magnitude seen in previous studies. A preliminary estimate of the expected number of IHD deaths suggested that there may be over 300 deaths from IHD in the study (Scottish Health Statistics, 1993. ISD Publications, Volume 35). The lower number of deaths seen is likely to be due to the relatively young age of the population presenting to EBU and to the fact that 10% of the population are breast cancer patients with a correspondingly higher breast cancer death rate.

For cause of death due to thromboembolism, the power calculations suggest that the number of cases available is sufficient to detect odds ratios of between 4 and 6 for exposed compared with non exposed individuals, for a correlation coefficient of exposure between controls and cases of 0.25 and 0.5 respectively. The WHO collaborative study of the risk of venous thromboembolic events associated with OC use showed an odds ratio of 4.15 (95% CI:3.09-5.57) for a hospital based case-

control study in Europe (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception, 1995). In this study, the prevalence of current OC use among the cases and controls was 61.2% and 34.1% respectively. The level of exposure in cases and controls is clearly an important factor in determining study power.

There are a number of factors in this study which compromise its statistical power to detect differences between exposed and non exposed individuals. Firstly, the number of cases and controls available for the analyses is less than the number identified as eligible for statistical analysis in the linked dataset and this is due to missing exposure data on the EBU database. Secondly, the level of exposure of the controls is relatively low at 20.3%. Maximum study power is obtained with control exposure levels of 40-50% (Breslow and Day). It is worthy of note that the exposure levels in cases (IHD –25%, thromboembolism-20%) does not differ greatly from that in controls (20.3%) and this results in a correlation coefficient of exposure between cases and controls which approaches unity.

The levels of oestrogen exposure observed in this study may reflect a true situation. However the possibility cannot be ignored that they might be the result of incorrect data entry and coding on the EBU database resulting in non-differential errors in exposure assessment. This would be the case if OC and HRT use had been recorded as NONE when the true value is MISSING. Problems with the classification of menstrual status on the database have already been identified and other errors cannot be ruled out. Further, it is known that during the time frame covered in this study (1989-1994), significant problems were experienced with the EBU computer system, particularly on two occasions when data were transferred from one system to another. Problems of system incompatibility resulted in loss of data during these transfer processes.

Thus, the lack of association between exogenous oestrogen exposure and death from ischaemic heart disease or thromboembolism seen in this study may be attributable to

a lack of power and is not necessarily indicative that such an association does not exist. The combining of HRT and OC use into a single exposure category may also dilute the results of the study; the literature appears to point to effects of exposure to HRT and OC acting in opposite directions. Whereas much of the published data on postmenopausal oestrogen use point to a protective effect, the evidence is clearly indicative of an increased risk of myocardial infarction associated with exposure to exogenous oestrogens as oral contraception. A further factor is that, according to published data, the effects seen are limited to current oestrogen use and by combining current and previous use into a single exposure category, any effects may be diluted out.

11.2 Recent published data of oestrogen exposure and cardiovascular disease

Although a lower rate of coronary heart disease in postmenopausal women who take oestrogen is well documented and discussed in *Chapter 8*, these data relate to observational studies and have not been confirmed in clinical trials. More recently, two large clinical trials have been published and the data challenge this premise. The HERS (Heart and Estrogen/progestin Replacement Study) study randomised 2,763 women with established coronary disease to combined OC or placebo. The main outcome measure was the occurrence of non fatal MI or death from coronary heart disease and over a follow-up of 4.1 years, there was no difference in the number of coronary events in the two groups. Further there was a statistically significant trend to more events occurring in the active treatment group in the first year (Hulley et al., 1998).

The second study involved women starting HRT with a pre-existing heart condition and suggests that women with previous heart disease should not be prescribed HRT. These data emerged from a further analysis of the 1996 CARS trial (Coumadin-Aspirin Reinfarction Study) which collected data on 8,803 heart disease patients including 1857 postmenopausal women. The results showed that of 111 women who started HRT after a heart attack, 32.5% were hospitalised for unstable angina within

a year. This compares with 21% of 413 women who had used hormones before their first attack and continued to use them during the study and 17% of 1,333 women who had never used hormones.

11.3 Suggestions for future work

11.3.1 Validation of exposure data

Based on the findings of the current study, a number of extensions to this work can be proposed. Firstly, it would be of value to audit the EBU database to determine the accuracy and validity of the data, particularly those data relating to oestrogen exposure. As has already been discussed, the source data (hospital case-notes) for this study tend not to be available for patients with benign disease who have died. However, notes for all cancer patients are available and checks in this group would be feasible and would allow the accuracy of the database to be audited against the source data. An estimate of the error rate in data recording for various parameters in this sample could be obtained. If this exercise confirmed the validity of the levels of oestrogen exposure recorded then it is clear that, because of the similar exposure levels in cases and controls, a considerable increase in the number of cases would be required to reliably detect an association. The number of events could be increased by increasing the length of follow-up and re-running the linkage procedure. Now that the linkage between the EBU database and the ISD combined database has been established and the methodology is in place, this is a straightforward operation. A modification to the method used to select controls in the current study, whereby controls are selected who are free of the endpoint when the case is diagnosed should be implemented in any future study.

11.3.2 Extensions to the current study

Provided that the validity of the EBU data was confirmed, a number of extensions to the current study can be proposed. Linkage of the EBU database has only been undertaken with SMR6 and GRO death records. This could be extended to include linkage with SMR1 enabling a comprehensive study of all morbidity, resulting in a period of hospitalisation, to be undertaken. Such an extension to the study is likely to

provide large numbers of cases but these could be adequately processed by the electronic methods that have been established. Potential therapeutic areas for study are discussed below.

Cognitive function

There are plausible mechanisms by which oestrogen may affect cognition but studies of the effects of oestrogen replacement on cognitive function and dementia in postmenopausal women have produced heterogeneous results. There have been positive results in studies of verbal memory and oestrogen has been shown to cause changes in brain activation patterns in regions of the brain associated with short-term memory function. The changes seen appear to reinstate the patterns seen in younger women (Shaywitz et al., 1999). A meta analysis of 10 observational studies investigating the risk of developing dementia suggested a 29% reduction associated with postmenopausal oestrogen use but concluded that further large studies are required. A recently published case-control study involving 222 cases and 222 matched controls has produced further evidence of a reduction in the risk of postmenopausal women developing Alzheimer's disease associated with hormone replacement therapy (odds ratio 0.42, 95% CI: 0.18-0.96, $p=0.04$) (Waring et al., 1999).

Fractures

There is no doubt that sustained administration of oestrogen to postmenopausal women maintains skeletal bone density and protects against fracture, although many questions remained to be answered about the optimal use of this treatment. A case-control study of 1,327 postmenopausal women with hip fracture and 3,262 controls, which was published in 1998, concluded that recent use of HRT is required for optimum fracture protection and that the protective effect increases with duration of use. Current users had an odds ratio of 0.35 (95% CI: 0.24-0.53) for hip fracture (Michaelsson et al., 1998). The same group have also published data showing a 25% reduction in the risk of hip fracture associated with OC use (OR 0.75, 95% CI: 0.59-

0.96) (Michaelsson K et al., 1999). Extension of the current linkage to include SMR1 would allow identification of those women admitted to hospital for fracture and would enable a study of the association of fracture with exposure to oral contraceptives and postmenopausal oestrogens.

Colorectal cancer

A number of studies have been published which suggest a reduced incidence of colorectal cancer in postmenopausal women receiving oestrogen replacement. In the current study, 79 colorectal cancers were identified and 60 of these have a date of onset of 1989 or later. The majority of these are likely to be eligible as cases (date of onset after date of presentation to EBU). Provided that the exposure data on the EBU database were verified, an analysis of these data would be of interest.

11.3.3 New study design

If source data verification of a sample of cases and controls indicated that the details of oestrogen exposure recorded on the EBU database are unreliable, then there is little to be gained from pursuing the extensions to the study discussed above. The question of morbidity and mortality associated with exogenous oestrogen exposure would best be addressed by a designing an alternative study. One option would be a prospective, cohort study with baseline data, including oestrogen exposure, being recorded at study entry (presentation to EBU). The same linkage methodology that has been established for the current study could be used to determine the number of cases occurring in exposed and non exposed individuals. The design of such a study is illustrated in *Chapter 9, Figure 9.1*. A prospective study of this type would require a number of years of follow-up but there would be some advantages in adopting this approach. For example, there would be the opportunity to set up methods for the reliable collection of all covariates at baseline, including smoking status, previous medical history and other risk factors. There would also be the opportunity to study HRT and OC as separate exposures, to look at the effect of duration of exposure and to look separately at current and previous exposure.

12.1 Study Conclusions

The results obtained from the updated data linkage of the Scottish adjuvant tamoxifen trial database with the ISD combined database provide further substantial evidence for the protective effect of current tamoxifen use against ischaemic heart disease and for an increased risk of thromboembolism associated with current use. The study has therefore achieved its aim of strengthening the evidence base of the risks and benefits associated with tamoxifen use in relation to these particular parameters, which are among the most intensively studied. The incidence of ocular toxicity associated with tamoxifen use has, in general, provoked less discussion than the cardiovascular effects but the results of this study, which indicate a substantially increased risk of cataract development with tamoxifen are particularly striking. The data obtained are in accordance with recent published data from the US Breast Cancer Prevention Trial (Fisher et al., 1998) and this is an area worthy of consideration when prescribing tamoxifen therapy for prolonged periods.

The data for the risk of fracture in this study does not provide clear evidence for a beneficial effect but does emphasise the need for further prospective studies of this complex area. The study provides further evidence for the endometrial carcinogenicity of long-term tamoxifen, although numbers are small and statistical evaluation was not feasible. Claims of carcinogenicity at other sites, such as colorectal, have not been substantiated.

The attempt to apply the data linkage methodology to answer related questions regarding the effects of exposure to exogenous oestrogens, using the Edinburgh Breast Unit database failed to produce informative results for reasons discussed in *Chapter 11*. Further, no attempt has been made to ascertain the risk of breast cancer development associated with oestrogen use. However, the study has established the methodology for data linkage of this database with SMR1, GRO records and also, although not used in the current study, SMR1. The study has also performed a

valuable audit of the Edinburgh Breast Unit database and opened the way for future studies.

12.2 New agents for hormone replacement therapy

Tamoxifen remains the treatment of choice as first-line endocrine therapy in women with breast cancer. As understanding of its mode of action increased, particularly with reference to its tissue specific actions as both an oestrogen agonist and antagonist, it was inevitable that the potential of agents with a similar profile of action would be investigated as possible hormone replacement therapies. The development of tamoxifen has been instrumental in the development of a new generation of pharmaceuticals offering significant potential benefits for women's health, beyond the treatment of breast cancer.

Although tamoxifen was itself never seriously considered as an oestrogen replacement therapy, such a role was tentatively discussed when evidence of its oestrogen agonist activity on the skeleton and cardiac system, together with its oestrogen antagonist action on the breast started to accrue. Here was a molecule which performed the functions of oestrogen on the skeleton and the heart but did not carry an increased risk of breast cancer, but rather may be associated with a reduced risk (Jackson, 1997). This led to a search for other candidate molecules and this search gathered pace as the evidence for the clinical benefit of tamoxifen in postmenopausal cardiac disease and loss of bone density became apparent. The potential of these compounds as long-term hormone replacement interventions to prevent some of the age-related problems seen in postmenopausal women as well as harnessing the potentially beneficial effect of chemoprevention of breast cancer is very significant.

12.3 Selective Oestrogen Receptor Modulators (SERMs)

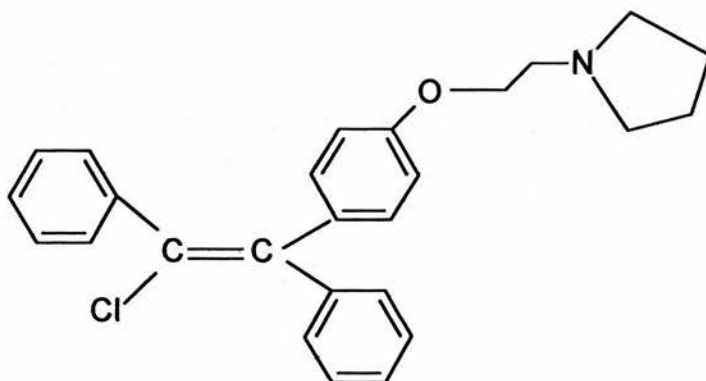
The group of compounds known as selective oestrogen receptor modulators, or SERMs, has been undergoing development over the past few years. These are a structurally diverse group of compounds which, like oestrogen, interact with the nuclear receptor but are distinguished from oestrogen by their ability to act as either an oestrogen agonist or antagonist depending on the target tissue and the hormonal milieu. The SERM profile is therefore distinct from that of pure oestrogens and pure oestrogen antagonists. SERMs of different structural types, such as tamoxifen and raloxifene, can be distinguished from each other by their different activity profile in specific tissues, for example in the uterus. It is the tissue selectivity of SERMs which has led to their being developed as oestrogen replacement therapies for the treatment of otherwise healthy, postmenopausal women.

12.4 SERMs as oestrogen replacement therapies

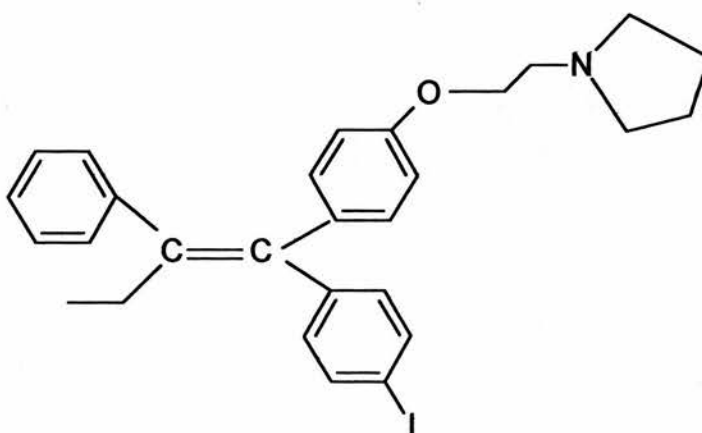
Hormone replacement therapy has the potential to prevent many of the diseases associated with oestrogen deficiency in postmenopausal women. However, there is a lack of data on optimum dose duration and, in particular, on when to initiate therapy. Currently, hormone replacement therapy is given as oestrogen or combined oestrogen-progestin preparations. Problems of unwanted side-effects result in lack of compliance and make them unsuitable for long-term use. There have been many studies investigating the risk of breast cancer associated with HRT use. The reanalysis in 1997 of 51 such studies reaffirmed that the risk of having breast cancer diagnosed is increased in women receiving HRT and the risk increases with increasing duration of use (Collaborative Group on Hormonal Factors in Breast Cancer, 1997). In the Nurses' Health study of long-term HRT, the lower cardiovascular mortality seen was cancelled out after 10-years treatment by an increase in breast cancer mortality (Grodstein et al., 1997). Continuing concern about the increased breast cancer risk means that many clinicians are reluctant to use oestrogen-based HRT in the preventative setting.

Two structural classes of SERMs have been identified – the triphenylethylene derivatives and the benzothiophene derivatives. The mechanisms by which these molecules elicit tissue-specific responses are currently the subject of intensive investigation. It is postulated that the structural differences may influence the conformations of their respective receptor-ligand complexes and this, in turn, may affect which oestrogen-responsive genes are modulated in various tissues (Grese et al., 1997).

Figure 12.1

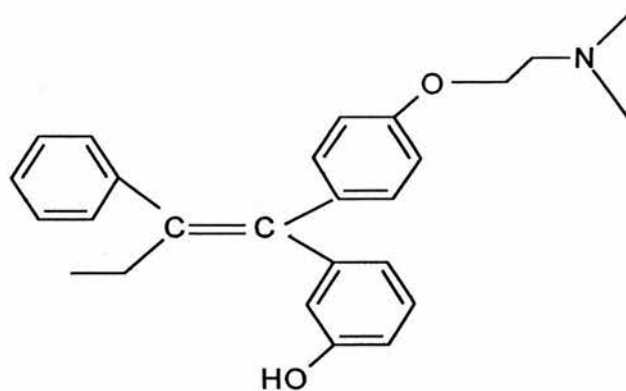


Clomiphene



Idoxifene

Figure 12.1 (continued)



Droloxifene

The structure of some triphenylethylene derivatives

12.5 Triphenylethylene derivatives

The triphenylethylene tamoxifen may be considered, in retrospect, a first-generation SERM because of its ability to exert antioestrogenic action on breast tissue and an agonist action on, for example, the cardiovascular system and skeleton. A number of triphenylethylene derivatives have subsequently been developed and the structure of some of these are shown in *Figure 12.1*. The best known of these is toremifene, which is a monochloro-derivative of tamoxifen, the structure of which has already been described in *Figure 7.1*. Toremifene is licensed as Fareston® for use in the treatment of advanced, tamoxifen-resistant breast cancer. However, as already described in *Chapter 7, Section 7.5*, toremifene does have a stimulatory effect on the endometrium and is associated with increased endometrial thickness. Early evidence also suggests that this molecule is less effective than tamoxifen in protecting against loss in bone mineral density in postmenopausal breast cancer patients (Marttunen et al., 1998). Toremifene is currently undergoing evaluation as a therapy for breast cancer in the adjuvant setting and as an agent for the prevention of breast cancer.

Studies in rats have shown a favourable activity profile for idoxifene with protection against bone loss, a reduction in total cholesterol and a lack of growth promoting activity in the uterus (Nuttall et al., 1998). However Phase III clinical trials for the prevention of osteoporosis were prematurely stopped because of the high number of gynaecological events, including endometrial thickening, pelvic organ prolapse and polyps, seen in an interim safety review after one year of therapy (SCRIP, No.2431, April 23rd 1999, Page 21). Phase II studies of idoxifene in the treatment of advanced breast cancer are continuing. Droloxifene is currently in Phase III trials for the prevention of osteoporosis. Cell culture work with this molecule suggests that induction of apoptosis may be a mechanism for both its oestrogen agonist effects in bone and its antagonist effects in breast tissue (Grasser et al., 1997).

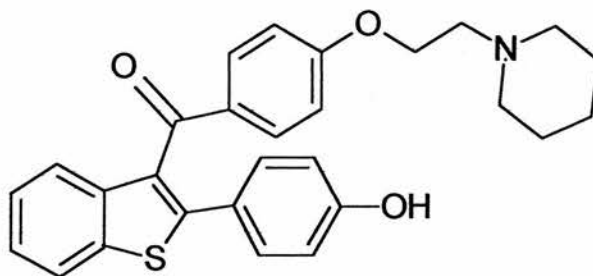
Another triphenylethylene derivative, clomiphene, is marketed as Serophene® and is used to initiate or augment ovulation by antagonising oestrogen at the hypothalamus and pituitary.

12.6 Benzothiophene derivatives

Raloxifene (LY139481 HCl) is a benzothiophene derivative and the structure is shown in *Figure 12.2*. This molecule was the first compound to be given the label of SERM and is the product in the most advanced stage of clinical development. Preclinical studies in ovariectomised rats showed that raloxifene can prevent bone loss and lower serum cholesterol, without stimulating proliferation of the endometrium (Black et al., 1994). This unique SERM profile encouraged investigation of its clinical use in healthy postmenopausal women as an oestrogen replacement therapy.

A clinical study involving 390 healthy, postmenopausal women showed that raloxifene favourably alters biochemical markers of biochemical risk, with reductions in LDL cholesterol, fibrinogen, lipoprotein a, and an increase in HDL₂ cholesterol (Walsh et al., 1998). A placebo controlled trial in 601 postmenopausal women also showed reductions in LDL and total cholesterol in the raloxifene group. This study also demonstrated significant increases, from baseline values, in BMD of the lumbar spine, hip and total body compared with the placebo group, in which there were decreases in BMD. These benefits were seen after 24 months of treatment and there was no evidence at any time during the study of stimulation of the endometrium and increased endometrial thickness (Delmas et al., 1997).

Figure 12.2



Raloxifene

The structure of a benzothiophene derivative

Raloxifene was launched in the UK in September 1998 for the prevention of postmenopausal osteoporosis and is marketed under the tradename of Evista. Meanwhile further long-term clinical studies are currently underway. The MORE study is looking at, amongst other parameters, the effect of raloxifene therapy on the incidence of bone fractures and its effectiveness in the prevention of breast cancer. Preliminary data have recently emerged from this study indicating that raloxifene may have a role in the treatment, as well as in the prevention, of osteoporosis. 6,828 women who had already developed osteoporosis were followed up with vertebral radiography at 36 months. At least one new vertebral fracture was found in 10.1% of the women given placebo, in 6.6% of those given 60 mg raloxifene daily and in 5.4% of those given 120 mg daily. The relative risk for spinal fracture in the low and high

dose groups compared with placebo were 0.7 (95% CI: 0.5-0.8) and 0.5 (95% CI: 0.4-0.7) respectively (Ettinger et al., 1999). This study has also reported on the incidence of breast cancer development. Thirteen cases of breast cancer have been confirmed among the 5,129 women randomised to raloxifene compared with 27 cases among 2,576 women randomised to placebo (RR 0.24, 95% CI: 0.13-0.44, $p < 0.001$) (Cummings et al., 1999). The main adverse effect reported within the MORE study is an increased risk of venous thromboembolism associated with raloxifene (Ettinger et al., 1999).

Two other large randomised studies of raloxifene are ongoing. In the STAR study raloxifene is being tested against tamoxifen as a chemopreventative agent in breast cancer and the RUTH study is investigating the cardiovascular effects of raloxifene.

12.7 Conclusions

Clearly, SERMs hold much promise in the amelioration of signs and symptoms of postmenopausal oestrogen deficiency. However much work still requires to be done to unleash the potential of these, so called, 'magic bullet' molecules and to develop the ideal hormone replacement therapy. The ideal SERM will have antioestrogenic effects in the breast and uterus and act as an oestrogen agonist on bone and lipids. Many pharmaceutical companies' attempts to find the ideal SERM have been unsuccessful. Pfizer has recently dropped development of droloxifene for breast cancer therapy, although it is still investigating the product for osteoporosis. SmithKline Beecham and GlaxoWellcome have also discontinued SERM development. Lilly is however developing a third generation SERM, arzoxifene, which has shown a 25,000-fold greater affinity for the oestrogen receptor than tamoxifen with no pathological endometrial thickening at any of the doses tested. Hopefully, the many studies currently ongoing will answer some of the questions and the new therapies being developed will gain widespread clinical acceptance by eliminating the undesirable side effects of therapy.

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Appendix 1

**McDonald CC, Stewart HJ, for the Scottish Breast Cancer
Committee**

Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial.

BMJ 1991;303:435-437

(reproduced with permission of the Scottish Cancer Trials Breast Group)

Fatal myocardial infarction in the Scottish adjuvant tamoxifen trial

C C McDonald, H J Stewart for the Scottish Breast Cancer Committee

Abstract

Objective—To investigate the incidence of fatal myocardial infarction in women in the two randomised arms of the Scottish adjuvant tamoxifen trial.

Design—Retrospective review of hospital notes to determine with the greatest possible certainty women who had died of an acute myocardial infarction.

Setting—Scottish Cancer Trials Office, the University of Edinburgh.

Patients—1070 postmenopausal women with operable breast cancer who were randomised to receive either adjuvant tamoxifen for five years or until relapse (539 patients) or tamoxifen for at least six weeks on the confirmation of first recurrence (531 patients).

Main outcome measures—Incidence of fatal myocardial infarction in women with no known or suspected systemic cancer.

Results—Of the 200 women who died in the adjuvant tamoxifen arm of the trial, 44 were free of cancer at death and 10 of these died of myocardial infarction. In the observation arm 251 women died, of whom 61 showed no evidence of systemic cancer and 25 had a fatal myocardial infarction. The incidence of fatal myocardial infarction in the two groups was significantly different ($\chi^2=6.88$, $p=0.0087$).

Conclusion—Tamoxifen given for at least five years as adjuvant therapy for breast cancer seems to have a cardioprotective oestrogen-like effect in postmenopausal women.

Introduction

Tamoxifen is a non-steroidal antioestrogen and is considered to be the front line endocrine treatment for breast cancer. It is widely used in postmenopausal women both as treatment for advanced disease^{1,2} and as adjuvant treatment in early disease, when it has been shown to delay recurrence and increase survival.³ Increasing numbers of premenopausal women are also now receiving tamoxifen as adjuvant treatment.⁴

Although tamoxifen was given for up to two years in most early clinical trials, evidence is growing that better results may be obtained with longer treatment.⁵ Studies in animals have shown that the effect of tamoxifen on breast carcinoma cells is tumourostatic rather than tumoricidal. The growth of MCF-7 tumour cells can be reactivated by supplementation with oestrogen after up to six months' treatment with tamoxifen,^{6,7} suggesting that long term or indefinite treatment should be advised. A trial has been proposed to investigate whether five years' treatment with tamoxifen has any preventive effect on the development of breast cancer in women considered to be at increased risk.

Increased length of exposure to tamoxifen raises the question what are the long term effects of the treatment.

Acute toxicity is known to be low,⁸ but the possibility that prolonged exposure may result in premature osteoporosis and cardiovascular disease as a result of antioestrogenic action must be considered, oestrogens being essential for maintaining bone density and a favourable lipid profile. Various oestrogen-like actions of tamoxifen have been shown, including effects on the liver resulting in increased concentrations of sex hormone binding globulin and thyroxine binding globulin.⁹ Laboratory studies have shown that tamoxifen has a favourable effect on bone density,¹⁰ and clinical results have been encouraging.^{11,12}

The Scottish adjuvant tamoxifen trial was set up to compare the effect on survival of adjuvant tamoxifen for five years with that of tamoxifen for recurrent breast cancer. We compared the incidence of fatal myocardial infarction in postmenopausal women randomised within this trial.

Patients and methods

Between 1978 and 1984, 1323 women with operable breast cancer were entered into four randomised controlled trials of adjuvant tamoxifen. Patients were randomised to receive tamoxifen either immediately after mastectomy (20 mg/day for five years or until confirmed relapse) or for a minimum of six weeks on confirmation of first recurrence. Eleven women were withdrawn from the trial within the first month because of major protocol violations, leaving 1312 evaluable cases. Fatal myocardial infarction was not recorded in any premenopausal woman, and this analysis is confined to the 1070 women who had not had a menstrual period for more than one year at entry to the study. Of these women, 539 were randomised to receive adjuvant tamoxifen and 531 to receive tamoxifen at first relapse.

Those patients who had not had a recurrence and were still taking tamoxifen after five years were considered eligible for rerandomisation to stop tamoxifen or to continue until relapse or death. As a result 176 patients continued treatment beyond five years, either after rerandomisation or electively.

Information on disease state, possible new primary tumours, and details of tamoxifen and other treatments was collected annually and at death. After a woman died the records were carefully checked, with particular importance being placed on establishing, with the greatest possible certainty, the cause of death. Any doubt about the presence or source (from breast or other confirmed or suspected primary tumour) of metastatic disease was recorded (table 1).

We analysed the results using Pearson's χ^2 test and a survival analysis in which women who had died of myocardial infarction were treated as events and women who had died of other causes or who were still alive were censored. The hazard ratio and 95% confidence interval were calculated with Cox's proportional hazards regression model.

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Table I shows the cause of death in the 451 women who died (200 from the adjuvant arm and 251 from the observation arm). Evidence from postmortem examination was available for 38 women. We restricted the analysis to those who had a fatal myocardial infarction in the absence of known or suspected systemic cancer.

Ten women in the adjuvant tamoxifen arm of the trial were recorded as having died of acute myocardial infarction. The median age of these women was 71 (range 65-77) years and the median duration of exposure to tamoxifen was 29 (9-93) months. Nine of the women were still receiving tamoxifen at the time of death and one had stopped treatment two months before her death.

TABLE I—Cause of death in 451 postmenopausal women in trials of adjuvant tamoxifen

Cause of death	Adjuvant arm (n=200)	Observation arm (n=251)
Breast cancer present at death:		
Breast cancer	137	163
Breast cancer and myocardial infarction	2	7
Breast cancer and vascular disease*	6	10
No breast cancer present at death:		
Myocardial infarction	10	25
Vascular disease*	14	13
Second primary cancer	12	15
Other causes	8	8
Disease state at death uncertain:		
Unproved systemic spread	6	7
Cancer but source uncertain	5	3

*Includes cerebrovascular accident, congestive cardiac failure, chronic ischaemic heart disease, and mitral valve disease.

In the observation arm 25 women died of acute myocardial infarction at a median age of 73 (59-80) years. Twenty one of these women had had no known exposure to tamoxifen. The four other women were all receiving tamoxifen when they died: one had received adjuvant tamoxifen for four months in error, two women had been successfully treated for local or regional recurrence with local therapy and tamoxifen for 12 and 13 months, and one woman had received tamoxifen for 14 months after excision of a contralateral primary breast tumour. Table II shows the distribution of duration of treatment of women in the adjuvant and observation arms.

TABLE II—Duration of treatment with tamoxifen in women who died of myocardial infarction in adjuvant and observation arms of trials

Duration of treatment (years)	Adjuvant arm (n=10)	Observation arm (n=25)
No treatment		21
<2	4	4
2-4	2	
>4	4	

Women in the adjuvant arm were significantly less likely to die of myocardial infarction than were women in the observation arm ($\chi^2=6.88$, $p=0.0087$). Women receiving adjuvant tamoxifen have a significantly increased survival¹³ and hence a longer time at risk of dying from a cause other than breast cancer. Thus, the beneficial effect of tamoxifen on myocardial infarction could be greater than is apparent from these data. Survival analysis using death from myocardial infarction as the end point confirms the significance of this result ($p=0.0054$, Mantel-Cox); the hazard ratio was 0.37 (95% confidence interval 0.18 to 0.77).

Twenty seven women died of vascular diseases, which included cerebrovascular accident, congestive cardiac failure, chronic ischaemic heart disease, and mitral valve disease. We found no difference in the

and observation arms of the trials.

Discussion

Tamoxifen exhibits a range of complex pharmacological properties, and may behave as an oestrogen or an antioestrogen depending on the target site.¹⁴

Studies in which the serum concentrations of lipids and lipoproteins in patients receiving tamoxifen have been monitored have shown an oestrogenic effect, with decreases in total and low density lipoprotein cholesterol concentrations and an increase in high density lipoprotein cholesterol concentration.^{15,16} Cholesterol and lipoproteins are implicated as risk factors for cardiovascular disease, and the authors of these studies conclude that tamoxifen exerts a favourable effect on the lipid profile over 12 months' treatment. Additional studies are needed to determine if the effect is maintained beyond 12 months' treatment and the effect of stopping tamoxifen.

We found a significant reduction in the incidence of fatal myocardial infarction in women receiving adjuvant tamoxifen, which provides clinical evidence to support the above biochemical findings. A similar reduction in deaths from myocardial infarction in postmenopausal women receiving oestrogen replacement therapy was observed in a study in southern California.¹⁴ Among 8841 women aged 44-101 years followed up for six years, 55 women who were receiving oestrogen therapy died of myocardial infarction compared with 94 women who were not given oestrogens (relative risk=0.59, $p=0.002$).¹⁴

Bourne *et al* have shown that administering transdermal oestradiol to postmenopausal women reduces arterial impedance and decreases vascular tone, which would be an alternative explanation for the effect of tamoxifen on myocardial infarction.¹⁹ Other mechanisms, such as effects on insulin metabolism, should not be overlooked.²⁰

Reduced concentrations of antithrombin III have been reported during treatment with tamoxifen,¹⁵ and Dahan *et al* have suggested that tamoxifen could increase the risk of thromboembolism.²¹ We found no such increased risk, and a recent study has shown that the decrease in antithrombin III concentration paralleled by a greater decrease in fibrinogen concentration²²; the ratio of fibrinogen to antithrombin III concentration is thus decreased and the risk of thrombosis reduced.

Further follow up may provide information on whether the reduced incidence of fatal myocardial infarction in the adjuvant arm reverts to that found in the observation arm in those women who stop treatment at five years. We are currently investigating whether adjuvant tamoxifen has a similar effect on the incidence of non-fatal myocardial infarction.

The members of the Scottish Breast Cancer Committee W D George (chairman), K Bartlett, U Chetty, J A Dev D Everington, O Eremin, Patrick Forrest, T Habesh R A Hawkins, A Hutcheon, D Kerr, R E Leake, R C Leonard, C S McArdle, M McCallum, C McDonald Preece, R Prescott, A Rodger, D C Smith, H J Stewart, P V Walsh.

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Survival with bladder cancer, evaluation of delay in treatment, type of surgeon, and modality of treatment

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Abstract

Objective—To determine whether length of delay before treatment; specialty and grade of the surgeon; and use made of surgery, radiotherapy, and chemotherapy influenced the survival of patients with cancer of the bladder, after adjusting for case severity.

Design—Retrospective cohort study.

Setting—South East and South West Thames health regions.

Patients—609 men aged under 75 resident in the South Thames regions who had been registered as new cases of bladder cancer in 1982, 35 of whom were excluded, leaving 574 eligible patients. Analysis was based on 75% retrieval rate for case notes.

Main outcome measures—Duration of survival from date of diagnosis of the bladder tumour.

Results—10 prognostic variables were used to adjust for case severity. The median delay from referral to first treatment was 48 (interquartile range 27-84) days. Treatment after a short delay was associated with shorter survival because of the early treatment of more severe cases. Consultants treated 68% of patients, trainee surgeons treated less severe cases. Initial treatment was by a urologist in 67% of cases, but the specialty of the surgeon was not associated with prognosis. The associations of radiotherapy, cystectomy, and systemic chemotherapy with survival were interpreted in terms of selection bias as well as therapeutic effect.

Conclusions—Case severity was the most important influence on survival and influenced length of delay before treatment, grade and specialty of the surgeon, and main treatment allocation. After adjusting for case severity, variations in these processes of care were not strongly associated with variations in survival.

Introduction

In England and Wales there are substantial variations in mortality from conditions which should be amenable to medical treatment^{1,2}; variations in survival with cancer have also been reported.^{3,4} Cancer of the bladder is one of the most common cancers,⁵ and health care is thought to influence the prognosis.⁶ An analysis

of cancer registry data for the South Thames regions showed systematic variation in the survival of patients with bladder cancer according to district health authority of residence (A Walker *et al*, unpublished data). One explanation for this observation could be that the quality of health care varied sufficiently to influence survival of these patients.⁷ The outcome of treatment for cancer of the bladder might be influenced by several characteristics of health care, including the length of delay before treatment⁸; the grade and specialty of the surgeon⁹; and the use made of surgery, radiotherapy, and chemotherapy.¹⁰ These were relevant factors to investigate, given current problems with long waiting times,¹¹ staffing and surgical specialties,¹² and organisation of cancer treatment services.¹³ This study aimed at determining whether, after allowing for the severity of the underlying disease, survival of patients with cancer of the bladder in the South Thames regions was influenced by these processes of care.

Patients and methods

The patients in the study were men aged below 75, resident in the South Thames regions, and registered with cancer of the bladder at the Thames Cancer Registry in 1982. The registry supplied a list of 609 names believed to fulfil the entry criteria. We excluded 35 men after examining their hospital records: seven in whom a diagnosis of bladder cancer had not been confirmed, four who were not resident in the South Thames regions at the time of diagnosis, and 24 whose disease was not first diagnosed in 1982. Thus 574 patients were eligible for further investigation. After consultants' approval had been obtained we abstracted data from the patients' hospital notes and radiotherapy records at 71 hospitals and 11 radiotherapy centres with standard data collection forms.

The patient's age was calculated as 1982 minus the year of birth and was included as a continuous variable. The presence of associated disease was noted from the record of the first hospital admission. The categories none, cardiac, respiratory, renal, and other (specify) were reduced for analysis to "comorbidity present" and "comorbidity absent." The histological extent of tumour invasion was classed according to the Inter-

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Appendix 2

**McDonald CC, Alexander FE, Whyte BW, Forrest AP, Stewart HJ,
for the Scottish Cancer Trials Breast Group**

**Cardiac and vascular morbidity in women receiving adjuvant
tamoxifen for breast cancer in a randomised trial.**

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Cardiac and vascular morbidity in women receiving adjuvant tamoxifen for breast cancer in a randomised trial

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Abstract

Objective—To determine any cardiac or vascular morbidity associated with long term treatment with tamoxifen given after mastectomy for primary breast cancer.

Design—Cohort study using linkage between database of a randomised trial and statistics of Scottish hospital inpatients to identify episodes of cardiac and vascular morbidity.

Setting—NHS hospitals in Scotland.

Subjects—1312 women who had undergone mastectomy for breast cancer and who were randomised either to a treatment group to receive adjuvant tamoxifen or to a control group to be given tamoxifen only on first relapse of disease. Maximum duration of tamoxifen treatment was 14 years. Total woman years of follow up were 9943.

Main outcome measures—Randomised and observational comparisons of risk (expressed as hazard ratios) of myocardial infarction, other cardiac event, cerebrovascular disease, or thromboembolic event according to treatment allocated and between non-users, former users, and current users of tamoxifen.

Results—Use of tamoxifen was associated with lower rates of myocardial infarction. Hazard ratio for women in control group was 1.92 (95% con-

fidence interval 0.99 to 3.73) compared with women allocated to adjuvant treatment. The association was stronger for current use: hazard ratio for non-users was 3.49 (1.52 to 8.03) compared with current users. Current users of tamoxifen, however, had higher rates of thromboembolic events: hazard ratio for non-users was 0.40 (0.18 to 0.90) compared with current users.

Conclusions—Our results provide further evidence that tamoxifen reduces the risk of myocardial infarction. Thromboembolic events should be carefully monitored in trials of tamoxifen, particularly those of prophylactic treatment, in which tamoxifen is given to healthy women.

Introduction

Tamoxifen, an oestrogen receptor antagonist, is widely used as an adjuvant treatment for primary cancer of the breast. The optimal duration of adjuvant treatment has not been established, and, although present indications suggest that five years of treatment is better than two years or less, it is possible that this should be life long. Several large trials of tamoxifen as a prophylactic treatment are also now under way. As these expose large numbers of healthy women to the

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drug, it is essential to assess the long term effects of treatment.

We previously reported that in the Scottish adjuvant tamoxifen trial women given long term adjuvant tamoxifen treatment showed a significant decrease in the number of deaths from myocardial infarction compared with those randomised for no adjuvant treatment.¹ There was no significant difference in the incidence of other fatal vascular events between the two arms of the trial.

In order to take account of non-fatal myocardial infarctions and other forms of cardiac and vascular disease, we now report the incidence of several potentially relevant events requiring admission to hospital in both arms of the trial population. These have been ascertained by linkage between the database of the tamoxifen trial and the Scottish Health Service computerised inpatient record scheme at the Information and Statistics Division. As this scheme records the diagnosis for all acute admissions to NHS hospitals in Scotland, we have been able to identify all relevant admissions to Scottish hospitals of trial patients after their mastectomy. Admissions to mental health institutions and obstetric units are not covered by the scheme.

Patients and methods

Between 1978 and 1984, 1312 eligible women with primary operable breast cancer were entered into the Scottish adjuvant tamoxifen trial.² Of these, 242 (18%) were premenopausal, being within one year of their last menstrual period. Women in the treatment arm of the trial received tamoxifen 20 mg daily for five years after their mastectomy (or until earlier relapse); women in the control arm did not receive adjuvant tamoxifen, but this was to be given for treatment of later relapse of disease. Women in the treatment arm who were alive and still free of relapse at five years were offered further randomisation either to stop tamoxifen treatment or to continue taking it until relapse or death. Information on recurrence and death was sought annually.

No baseline data were available for factors not considered relevant to survival from breast cancer. In particular information on smoking, blood lipids, weight, and blood pressure (important factors in cardiovascular morbidity) was not collected.

In designing the present study, we decided to include all eligible women rather than just postmenopausal women as previously¹ because our intention was to examine a range of potential health related effects and the inclusion of younger women would not increase the incidence of myocardial infarction itself.

DATA LINKAGE

A computerised linkage program at Information and Statistics Division attempted to identify episodes in the computerised inpatient record scheme for all trial patients for the period from 1 January 1978 to 31 December 1992. Surname, forename, date of birth, date of mastectomy, and date of death (if appropriate) were used as matching items. A probability based score was used to measure the likelihood of two records matching. The odds of a correct match were calculated for each variable and multiplied together to give the

overall probability that the two records belonged to the same person. The threshold value was kept low to minimise the risk of true matches being rejected.

In the Scottish cancer trials office each matching pair of records was checked. For each confirmed match, the date of admission and code of cause³ (other than those related to breast cancer) were added to the existing trial database. This included dates of patients' birth, randomisation, local and systemic relapse, and death. Allocated treatment and its duration were entered for all trial subjects. From the resulting database all the above items except locoregional relapse alone were extracted for the analysis. Table I shows the causes of hospital admissions considered in this report. These categories were defined before the data were inspected. Because of the small numbers involved, deep vein thrombosis and pulmonary embolus were analysed as a single group of thromboembolic events. Most of the subjects admitted to hospital for both deep vein thrombosis and pulmonary embolism had identical dates for the two; when they differed the earlier of the two dates was taken as the date of the thromboembolic event.

STATISTICAL ANALYSIS

To avoid the possible confounding effect of systemic recurrence of breast cancer and consequent treatment, each subject was considered to be at risk for the analysed events from the date of randomisation up to, but not beyond, that of any systemic recurrence.

Statistical evaluation was by the Cox proportional hazards method using the EGRET package, with censoring at date of systemic relapse, death, or at follow up to 31 December 1992. Results are given as hazard ratios and 95% confidence intervals. A hazard ratio greater than 1 indicates a benefit from tamoxifen, which is significant if the confidence interval does not overlap unity. All analyses were adjusted for age at entry.

TABLE II—Woman years at risk in treatment and control arms of study by exposure to tamoxifen

Woman years at risk*	Treatment arm (n=661)	Control arm (n=651)
Before use of tamoxifen†	224	3982
While using tamoxifen	3750	431
After stopping tamoxifen use	1457	99
Total	5431	4512

*Time from entry to trial to end of follow up period or to first systemic relapse.

†This is "never used tamoxifen" category.

The primary analysis investigated the main effect of treatment allocated by randomisation. As this did not take account of stopping treatment in the treatment arm or giving tamoxifen for locoregional relapse in controls (table II), additional analyses were carried out with time dependent covariates to examine risk according to actual tamoxifen use. Specifically, two factors were introduced: firstly, for comparison of women who never used tamoxifen with those who ever used it, women took the value 1 (never) until they were prescribed tamoxifen, after which the value changed to 2 (ever). The second factor, for current use of tamoxifen, had the value 2 at times of prescribed tamoxifen treatment (current user) and 1 at other times (not a current user). The values of these factors were known at all times for women in the trial. A third factor, the results of which are not reported in detail, took three values (never, current user, former user) defined in a similar way. Since the number of events available for analysis was small, the potential for checking the model's assumptions was limited. Details of the complexity of time dependent covariate analysis have been described elsewhere.⁴

TABLE I—Causes of hospital admissions included in study and numbers of cases analysed and of cases rejected because of presence of systemic cancer

International Classification of Diseases, Ninth Revision ³		No of cases	
Description	Code	Analysed	Excluded
Myocardial infarction	410	37	9
Cerebrovascular disease	430-437	45	7
Pulmonary embolism or phlebitis and thrombophlebitis	4151, 451	25	10
Other ischaemic heart diseases	411-414	42	10

Results

Subjects in the two arms of the study were well matched for age and menstrual status. The mean age in the treatment arm was 58.6 years (range 30-79) while that in the control arm was 59.1 years (27-79). In the treatment arm 18.5% of women were premenopausal, and 18.4% were premenopausal in the control arm.²

HOSPITAL ADMISSIONS

The incidence of hospital admission for myocardial infarction (fatal and non-fatal) was lower in the treatment arm of the study than in the control arm, and this difference was of borderline significance (table III, fig 1). There was also an apparent reduction in the incidence of other ischaemic cardiac episodes in the treatment arm, but this did not reach significance. There was a slight increase in the number of hospital admissions for thromboembolic events in the treatment arm, but there were no differences in admissions for cerebrovascular events (table III, fig 1).

TABLE III—Number and rate of adverse events and hazard ratio for women in control arm of study compared with those in treatment arm

Event	No of events (crude rate/1000 years at risk)		Hazard ratio (95% confidence interval)	P value
	Treatment arm (n=661)	Control arm (n=651)		
Myocardial infarction	14 (2.6)	23 (5.1)	1.92 (0.99 to 3.73)	0.051
Cerebrovascular disease	25 (4.6)	20 (4.4)	0.96 (0.53 to 1.73)	0.89
Thromboembolic event*	15 (2.8)	10 (2.2)	0.76 (0.34 to 1.70)	0.50
Other ischaemic heart disease	18 (3.3)	24 (5.3)	1.56 (0.85 to 2.87)	0.15

*Deep vein thrombosis or pulmonary embolism.

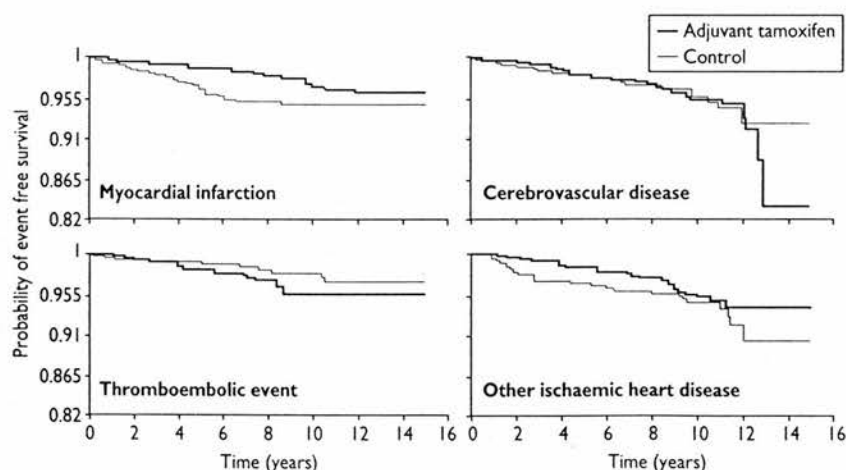


FIG 1—Survival curves for women randomised to adjuvant tamoxifen treatment or to control for the four study end points

TABLE IV—Number and rate of adverse events and hazard ratio for women who never used tamoxifen compared with those who ever used it

Event	No of events (crude rate/1000 years at risk)		Hazard ratio (95% confidence interval)	P value
	Ever used tamoxifen	Never used tamoxifen		
Myocardial infarction	15 (2.6)	22 (5.2)	2.03 (1.05 to 3.92)	0.033
Cerebrovascular disease	28 (4.9)	17 (4.0)	0.87 (0.47 to 1.59)	0.65
Thromboembolic event*	17 (3.0)	8 (1.9)	0.61 (0.26 to 1.42)	0.24
Other ischaemic heart disease	20 (3.5)	22 (5.2)	1.55 (0.84 to 2.84)	0.16

*Deep vein thrombosis or pulmonary embolism.

TABLE V—Number and rate of adverse events and hazard ratio for women who were not current users of tamoxifen compared with current users

Event	No of events (crude rate/1000 years at risk)		Hazard ratio (95% confidence interval)	P value
	Current users	Not current user*		
Myocardial infarction	7 (1.7)	30 (5.2)	3.49 (1.52 to 8.03)	0.001
Cerebrovascular disease	15 (3.6)	30 (5.2)	1.31 (0.69 to 2.46)	0.40
Thromboembolic event†	15 (3.6)	10 (1.9)	0.40 (0.18 to 0.90)	0.025
Other ischaemic heart disease	12 (2.9)	30 (5.2)	1.73 (0.87 to 3.43)	0.10

*Never used tamoxifen or former user.

†Deep vein thrombosis or pulmonary embolism.

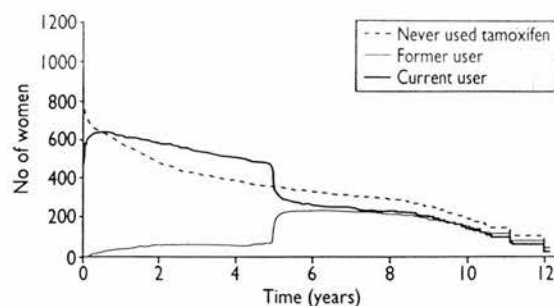


FIG 2—Numbers of women by category of tamoxifen use and time since randomisation

TAMOXIFEN USE

The patterns of tamoxifen use were complex (fig 2). In the event of a relapse of disease women in the treatment arm stopped taking tamoxifen whereas women in the control arm started taking tamoxifen. In addition a secondary randomisation altered the duration of tamoxifen use in the treatment arm.

When actual tamoxifen use was taken into account, the effect on risk of myocardial infarction was increased for the comparison of those who had ever used tamoxifen with those who had never used it (hazard ratio 2.03 (95% confidence interval 1.05 to 3.92)) (table IV) and for the comparison of current users with not current users (hazard ratio 3.49 (1.52 to 8.03)) (table V). Further analysis, comparing all three categories of tamoxifen use, showed that patients who had never received tamoxifen had a risk of hospitalisation for myocardial infarction (hazard ratio 3.36 (1.43 to 7.88) compared with current users) similar to that of former users (4.10 (1.39 to 12.13)).

THROMBOEMBOLIC EVENTS

Hospital admissions for the thromboembolic events of deep vein thrombosis and pulmonary embolus were increased in tamoxifen users, but this reached significance only for comparison of current users with not current users (table V).

The 15 current users who had a thromboembolic event ranged in age from 42 to 78 years (median 67) and had been receiving tamoxifen for between one and 134 months (median 62). Four of the women had been premenopausal at the time of starting tamoxifen treatment and had taken tamoxifen for 11, 38, 62, and 97 months.

Checking hospital discharge data for the six months before the thromboembolic event revealed that eight of the 25 cases had had inpatient surgery during this time. The time between surgery and the thromboembolic event was between three and seven weeks. Six of the eight patients were current users of tamoxifen, and two were in the control group. A ninth patient, a former user, had metastatic colon cancer. The results of reanalysis with these nine cases excluded showed no increased risk for women randomised to tamoxifen treatment and a smaller effect, with loss of statistical significance, in the comparison of current users with not current users.

Discussion

The primary comparison of the effects of allocated tamoxifen treatment (table III, fig 1) has greatest reliability because the equivalence of the groups on unmeasured baseline factors should be ensured by randomisation, but the effect of tamoxifen may be diluted by failure to take account of subsequent changes in its use. The analyses using time dependent covariates take account of this factor but, being observational in nature, are less reliable. We do not know that the women being compared in these analyses have equal distributions of risk factors and we acknow-

Key messages

- Increasing use of tamoxifen as adjuvant treatment for breast cancer and its use as a prophylactic in clinical trials highlight the need to determine the consequences of long term treatment
- In this study the database for the Scottish adjuvant tamoxifen trial was matched with records of inpatients in Scottish hospitals
- Tamoxifen treatment resulted in significant reduction in incidence of hospital admission for myocardial infarction
- There was some evidence that tamoxifen increased risk of pulmonary embolism
- The benefits of tamoxifen treatment for breast cancer outweigh any adverse effects, but healthy women in clinical trials should be monitored for thromboembolic events

ledge that the absence of information on smoking and blood pressure is a limitation, but we have no reason to suppose that these are associated with the decision to change treatment. The main reason for the change of status of women remaining in the analysis was locoregional relapse of disease which occurred more often in women in the control arm.² Treatment other than tamoxifen could possibly increase the risk of cardiovascular morbidity, and the observational comparisons may therefore underestimate the benefit for myocardial infarction but could overemphasise the risk of deep vein thrombosis and pulmonary embolism.

CARDIOVASCULAR AND CEREBROVASCULAR MORBIDITY

Our results indicate a protective effect of tamoxifen against hospital admission for acute myocardial infarction. This protection was greatest in current users and so may be lost when tamoxifen treatment is stopped. This is consistent with our previous report of fatal episodes. Our findings are also in keeping with reports that concentrations of plasma cholesterol and its low density lipoprotein fraction are lower in current tamoxifen users than in former and non-users.⁵⁻⁸ These factors are known to be related to coronary artery disease in men.⁹

A study of cardiovascular morbidity in 2365 patients in the Swedish trial of adjuvant tamoxifen reported a significant reduction of all cardiac disease in patients receiving tamoxifen,¹⁰ but there was no apparent decrease in either the myocardial infarction or ischaemic heart disease subgroup. As well as showing a reduction in myocardial infarction, our data suggest that ischaemic heart disease may have been reduced in the tamoxifen users. We found little association of tamoxifen use with cerebrovascular events (cerebral haemorrhage and cerebral thrombosis). Analysis of all cerebrovascular events in the Swedish data (similar to our cerebrovascular disease category) also failed to show an effect (Rutqvist, personal communication).

THROMBOEMBOLIC EVENTS

The Swedish workers failed to find a difference in the incidence of "thromboembolic disease" between patients receiving adjuvant tamoxifen and controls. They included cerebral thrombosis in this category. In our separate analysis of the effect of tamoxifen on the specific thromboembolic events of deep vein thrombosis and pulmonary embolism, the patients taking tamoxifen seemed to be at greater risk, but this was significant only in the analysis by current use (table V).

Our finding clarifies the effect of tamoxifen described in a retrospective review of venous and arterial thromboembolic complications in seven consecutive trials of adjuvant treatment for breast cancer conducted by the Eastern Cooperative Oncology Group.¹¹ This indicated a substantially greater number

of events in premenopausal patients receiving chemotherapy and tamoxifen than in those receiving chemotherapy alone. It has been suggested that a decrease in levels of antithrombin III in women treated with tamoxifen may be one factor contributing to hypercoagulability.¹²

Unlike in the above review, we censored for systemic relapse in our analysis, reducing the likelihood that the observed increase in thromboembolic events was related to breast cancer. Nine thromboembolic events occurred in patients who might be considered to have been at increased risk due to recent surgery or similar factors, but the time gap of 3-7 weeks between the two events decreases the likelihood of a direct relation. We could not identify all those who had had additional surgery without experiencing a thromboembolic event.

The numbers of subjects in our study are small, and the results concerning deep vein thrombosis and pulmonary embolism (our thromboembolic disease) should be interpreted with caution. However, they indicate that the incidence of thromboembolic events within tamoxifen prevention trials should be carefully monitored and that women entering these trials must be informed of this potential risk.

CONCLUSION

It is important to appreciate that our results do not cast doubt on the evidence that adjuvant tamoxifen is beneficial to women with breast cancer. In addition, our results and those which we have previously reported concerning fatal myocardial infarction¹ demonstrate an additional benefit for users of tamoxifen which is greater than any possible harmful thromboembolic effects.

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(Accepted 21 July 1995)

Appendix 3

Microsoft Access SQL queries

Ascertainment of Events

```
SELECT DISTINCTROW tamvalid.Surname, tamvalid.Initial, tamvalid.DOB,  
tamvalid.Event_date, cause_1, cause_2, cause_3, cause_4, cause_5,  
cause_6
```

```
FROM tamvalid
```

```
WHERE (((tamvalid.Cause_1) like '-414?') AND ((tamvalid.Score)>=17)) OR  
(((tamvalid.Score)>=17) AND ((tamvalid.Cause_2) like '-414?')) OR  
(((tamvalid.Score)>=17) AND ((tamvalid.Cause_3) like '-414?')) OR  
(((tamvalid.Score)>=17) AND ((tamvalid.Cause_4) like '-414?')) OR  
(((tamvalid.Score)>=17) AND ((tamvalid.Cause_5) like '-414?')) OR  
(((tamvalid.Score)>=17) AND ((tamvalid.Cause_6) like '-414?'));
```

```
SELECT DISTINCTROW Surname, DOB, Score, Misc, Event_date
```

```
FROM tamvalid
```

```
WHERE Score>17 AND Misc LIKE '*6' AND event_date LIKE '*150*'
```

```
OR Score>17 AND Misc LIKE '*9' AND event_date LIKE '*150*';
```


Appendix 4

Structure of Tambase database

Properties

Date Created:	07-Oct-98 9:00:27 AM	Def. Updatable:	True
Last Updated:	07-May-99 4:08:20 PM	OrderByOn:	False
RecordCount:	1312		

Columns

Name	Type	Size
TRLNO	Text	7
AllowZeroLength: False Attributes: Variable Length Collating Order: General ColumnHidden: False ColumnOrder: Default ColumnWidth: Default Description: Tamoxifen trial number DisplayControl: Text Box Ordinal Position: 1 Required: False Source Field: TRLNO Source Table: Tambase		
DOB	Date/Time	8
AllowZeroLength: False Attributes: Fixed Size Collating Order: General ColumnHidden: False ColumnOrder: Default ColumnWidth: Default Description: Date of birth Format: dd/mm/yy Ordinal Position: 2 Required: False Source Field: DOB Source Table: Tambase		
DRAND	Date/Time	8
AllowZeroLength: False Attributes: Fixed Size Collating Order: General ColumnHidden: False ColumnOrder: Default ColumnWidth: Default Description: Date of randomisation Format: dd/mm/yy Ordinal Position: 3 Required: False Source Field: DRAND Source Table: Tambase		

AGE		Number (Integer)	2
	AllowZeroLength:	False	
	Attributes:	Fixed Size	
	Collating Order:	General	
	ColumnHidden:	False	
	ColumnOrder:	Default	
	ColumnWidth:	Default	
	Decimal Places:	Auto	
	Default Value:	0	
	Description:	Calculated age	
	DisplayControl:	Text Box	
	Ordinal Position:	4	
	Required:	False	
	Source Field:	AGE	
	Source Table:	Tambase	
OPTION		Text	50
	AllowZeroLength:	False	
	Attributes:	Variable Length	
	Collating Order:	General	
	ColumnHidden:	False	
	ColumnOrder:	Default	
	ColumnWidth:	Default	
	Description:	Randomised option 1=tamoxifen 2=observation	
	DisplayControl:	Text Box	
	Ordinal Position:	6	
	Required:	False	
	Source Field:	OPTION	
	Source Table:	Tambase	
DMR		Date/Time	8
	AllowZeroLength:	False	
	Attributes:	Fixed Size	
	Collating Order:	General	
	ColumnHidden:	False	
	ColumnOrder:	Default	
	ColumnWidth:	Default	
	Description:	Date of metastatic relapse	
	Format:	dd/mm/yy	
	Ordinal Position:	7	
	Required:	False	
	Source Field:	DMR	
	Source Table:	Tambase	
DD		Date/Time	8
	AllowZeroLength:	False	
	Attributes:	Fixed Size	
	Collating Order:	General	
	ColumnHidden:	False	
	ColumnOrder:	Default	
	ColumnWidth:	Default	
	Description:	Date of death	
	Format:	dd/mm/yy	

	Ordinal Position:	8		
	Required:	False		
	Source Field:	DD		
	Source Table:	Tambase		
DATEMI			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Date of MI		
	Format:	dd/mm/yy		
	Ordinal Position:	9		
	Required:	False		
	Source Field:	DATEMI		
	Source Table:	Tambase		
DATECVA			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Date of CVA		
	Format:	dd/mm/yy		
	Ordinal Position:	10		
	Required:	False		
	Source Field:	DATECVA		
	Source Table:	Tambase		
DATEPE			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Date of PE		
	Format:	dd/mm/yy		
	Ordinal Position:	11		
	Required:	False		
	Source Field:	DATEPE		
	Source Table:	Tambase		
DATEDVT			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		

	ColumnWidth:	Default		
	Description:	Date of DVT		
	Format:	dd/mm/yy		
	Ordinal Position:	12		
	Required:	False		
	Source Field:	DATEDVT		
	Source Table:	Tambase		
D_FRACT			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Date of fracture		
	Format:	dd/mm/yy		
	Ordinal Position:	13		
	Required:	False		
	Source Field:	D_FRACT		
	Source Table:	Tambase		
S_FRACT			Text	50
	AllowZeroLength:	False		
	Attributes:	Variable Length		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Site of fracture		
	DisplayControl:	Text Box		
	Ordinal Position:	14		
	Required:	False		
	Source Field:	S_FRACT		
	Source Table:	Tambase		
TSTART			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Date tamoxifen started		
	Format:	dd/mm/yy		
	Ordinal Position:	15		
	Required:	False		
	Source Field:	TSTART		
	Source Table:	Tambase		
TSTOP			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		

Collating Order: General
ColumnHidden: False
ColumnOrder: Default
ColumnWidth: Default
Description: Date tamoxifen stopped
Format: dd/mm/yy
Ordinal Position: 16
Required: False
Source Field: TSTOP
Source Table: Tambase

D_CARDIAC Date/Time 8

AllowZeroLength: False
Attributes: Fixed Size
Collating Order: General
ColumnHidden: False
ColumnOrder: Default
ColumnWidth: Default
Description: Date of IHD
Format: dd/mm/yy
Ordinal Position: 17
Required: False
Source Field: D_CARDIAC
Source Table: Tambase

D_FUP Date/Time 8

AllowZeroLength: False
Attributes: Fixed Size
Collating Order: General
ColumnHidden: False
ColumnOrder: Default
ColumnWidth: Default
Description: Date of last follow-up
Format: dd/mm/yy
Ordinal Position: 18
Required: False
Source Field: D_FUP
Source Table: Tambase

D_CANCER Date/Time 8

AllowZeroLength: False
Attributes: Fixed Size
Collating Order: General
ColumnHidden: False
ColumnOrder: Default
ColumnWidth: Default
Description: Date of new primary cancer
Format: dd/mm/yy
Ordinal Position: 19
Required: False
Source Field: D_CANCER
Source Table: Tambase

S_CANCER	Number (Integer)	2
AllowZeroLength:	False	
Attributes:	Fixed Size	
Collating Order:	General	
ColumnHidden:	False	
ColumnOrder:	Default	
ColumnWidth:	Default	
Decimal Places:	Auto	
Default Value:	0	
Description:	Site of new primary cancer	
DisplayControl:	Text Box	
Ordinal Position:	20	
Required:	False	
Source Field:	S_CANCER	
Source Table:	Tambase	
D_ENDO	Date/Time	8
AllowZeroLength:	False	
Attributes:	Fixed Size	
Collating Order:	General	
ColumnHidden:	False	
ColumnOrder:	Default	
ColumnWidth:	Default	
Description:	Date of benign endometrial event	
Format:	dd/mm/yy	
Ordinal Position:	21	
Required:	False	
Source Field:	D_ENDO	
Source Table:	Tambase	
D_OCULAR	Date/Time	8
AllowZeroLength:	False	
Attributes:	Fixed Size	
Collating Order:	General	
ColumnHidden:	False	
ColumnOrder:	Default	
ColumnWidth:	Default	
Description:	Date of ocular event	
Format:	dd/mm/yy	
Ordinal Position:	22	
Required:	False	
Source Field:	D_OCULAR	
Source Table:	Tambase	
D_DEMENT	Date/Time	8
AllowZeroLength:	False	
Attributes:	Fixed Size	
Collating Order:	General	
ColumnHidden:	False	
ColumnOrder:	Default	
ColumnWidth:	Default	
Description:	Date of dementia	
Format:	ddVmmVyy	

	Ordinal Position:	23		
	Required:	False		
	Source Field:	D_DEMENT		
	Source Table:	Tambase		
comment			Text	50
	AllowZeroLength:	False		
	Attributes:	Variable Length		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	DisplayControl:	Text Box		
	Ordinal Position:	24		
	Required:	False		
	Source Field:	comment		
	Source Table:	Tambase		
d_censor			Date/Time	8
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Description:	Date censored		
	Format:	ddVmmVyy		
	Ordinal Position:	25		
	Required:	False		
	Source Field:	d_censor		
	Source Table:	Tambase		
start			Number (Long)	4
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Decimal Places:	Auto		
	Default Value:	0		
	Description:	Days from randomisation to tamoxifen started		
	DisplayControl:	Text Box		
	Ordinal Position:	26		
	Required:	False		
	Source Field:	start		
	Source Table:	Tambase		
stop			Number (Long)	4
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		

	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Decimal Places:	Auto		
	Default Value:	0		
	Description:	Days from randomisation to tamoxifen stopped		
	DisplayControl:	Text Box		
	Ordinal Position:	27		
	Required:	False		
	Source Field:	stop		
	Source Table:	Tambase		
event			Number (Long)	4
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Decimal Places:	Auto		
	Default Value:	0		
	Description:	1=Event 2=Censored		
	DisplayControl:	Text Box		
	Ordinal Position:	28		
	Required:	False		
	Source Field:	event		
	Source Table:	Tambase		
survtime			Number (Long)	4
	AllowZeroLength:	False		
	Attributes:	Fixed Size		
	Collating Order:	General		
	ColumnHidden:	False		
	ColumnOrder:	Default		
	ColumnWidth:	Default		
	Decimal Places:	Auto		
	Default Value:	0		
	Description:	Survival time (days)		
	DisplayControl:	Text Box		
	Ordinal Position:	29		
	Required:	False		
	Source Field:	survtime		
	Source Table:	Tambase		

Appendix 5

Microsoft Access update queries

DETERMINATION OF EVENT OR CENSORING

update TAM_(event) set EVENT = 0
where D_(event) is null
or D_(event) is not null and D_CENSOR ≤ D_(event);

update TAM-(event) set EVENT = 1
where D_(event) is not null and D_CENSOR > D_(event);

update TAMBASE set D_CENSOR = DMR
where DMR is not null;

update TAMBASE set D_CENSOR = DD
where DMR is null
and DD is not null;

update TAMBASE set D_CENSOR = D_FUP
where DD is null
and DMR is null;

update TAMBASE set D_CENSOR = datevalue('31/03/96')
where D_CENSOR > datevalue ('31/03/96');

DETERMINATION OF CENSORING DATE

update TAMBASE set D_CENSOR = DMR

where DMR is not null;

update TAMBASE set D_CENSOR = DD

where DMR is null

and DD is not null;

update TAMBASE set D_CENSOR = D_FUP

where DD is null

and DMR is null;

update TAMBASE set D_CENSOR = datevalue('31/03/96')

where D_CENSOR > datevalue ('31/03/96');

DETERMINATION OF TIME FROM RANDOMISATION TO TAMOXIFEN START/STOP

update TAMBASE set START = TSTART-DRAND;

update TAMBASE set START = 9999
where START is null;

update TAMBASE set START = 0
where START < 0;

update TAMBASE set STOP = TSTOP-DRAND;

update TAMBASE set STOP = 9999
where STOP is null;

Appendix 6

**Case-control data for ischaemic heart disease prepared for
conditional logistic regression analysis**

diagnosis	age strata	matching	oestrogen	outcome_ihd
2	5	10	11	
2	5	10	21	
1	6	11	21	
1	6	11	21	
1	6	11	20	
1	6	11	20	
1	6	11	11	
1	6	11	10	
1	6	11	20	
1	6	11	10	
1	6	11	10	
1	6	11	11	
1	6	11	21	
1	6	11	10	
1	6	11	20	
1	6	11	20	
1	6	11	20	
1	6	11	20	
2	6	12	21	
2	6	12	21	
2	6	12	10	
2	6	12	11	
2	6	12	20	
2	6	12	20	
2	6	12	20	
2	6	12	20	
2	6	12	20	
2	6	12	20	
2	6	12	10	
2	6	12	11	
2	6	12	20	
2	6	12	10	
2	6	12	20	
2	6	12	20	
2	6	12	20	
2	6	12	11	
2	6	12	10	
1	7	13	21	
1	7	13	20	
1	7	13	20	
1	7	13	10	
1	7	13	20	
1	7	13	20	
1	7	13	20	
1	7	13	21	
1	7	13	20	
1	7	13	21	
1	7	13	20	
1	7	13	11	
1	7	13	20	
1	7	13	20	

Appendix 7

**Sample of case-control data for thromboembolism prepared for
conditional logistic regression analysis**

diagnosis	age strata	matching	oestrogen	outcome_t
1	10	19	20	
1	10	19	20	
1	8	15	20	
1	10	19	20	
1	8	15	20	
1	7	13	20	
1	7	13	20	
1	11	21	20	
1	11	21	20	
2	11	22	20	
1	9	17	21	
1	9	17	21	
2	9	18	21	
1	10	19	21	
2	8	16	21	
1	10	19	21	
2	5	10	21	
1	7	13	21	
1	11	21	21	
1	10	19	21	
1	11	21	21	
2	4	8	11	
2	4	8	11	
1	10	19	21	
1	10	19	21	
1	8	15	21	
1	3	5	21	
2	2	4	21	
1	7	13	21	
2	7	14	21	
1	8	15	21	
1	7	13	21	
2	5	10	11	
2	11	22	21	
1	10	19	21	
1	6	11	11	
2	11	22	21	
1	10	19	20	
1	10	19	20	
1	11	21	20	
1	10	19	20	
1	4	7	11	
1	5	9	11	
1	9	17	21	
2	10	20	21	
1	7	13	21	
2	7	14	21	
1	10	19	21	
1	5	9	10	
1	6	11	20	